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# Interrelationship of thyroxine, exercise, and feed intake on serum and hepatic cholesterol levels

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LEVELS.

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Interrelationship of thyroxine, exercise, and feed intake  
on serum and hepatic cholesterol levels

by

Herbert Kunio Naito

A Dissertation Submitted to the  
Graduate Faculty in Partial Fulfillment of  
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DEDICATION

To my wife, Jane



## INTRODUCTION

Evidence for the salubrious effects of physical activity in decreasing serum cholesterol levels and in the possible retardation or prevention of the development of coronary atherosclerosis comes from epidemiological research (Holloszy et al., 1964; Fox and Skinner, 1964; Kannel, 1966), human experimental research (Golding, 1961; Campbell, 1966; Carlson and Fröberg, 1967), and animal experimental research (Montoye et al., 1962; Jones and co-workers, 1964; Gollnick and Simmons, 1967). While such investigators have found a significant relationship between physical activity and reductions in the serum cholesterol levels, others have found none (Carlson and Mossfeldt, 1964; Malhotra, 1967; Heyden, 1969).

Such conflicting and confusing reports are the results of (i) varying intensity and duration of physical activity, (ii) varying dietary composition, especially that of total calories, total fat, polyunsaturated and saturated fats, cholesterol, and dietary supplementation such as that of bile acids and goitrogens; and (iii) variation in the types and the intensity of "stress" to which the laboratory animal or man was subjected in the training period. Epidemiological research in particular is influenced by all three factors to a greater degree than laboratory experimentation, resulting in inconsistent findings. The more consistent laboratory investigations tend to support the hypothesis that exercise has a

hypocholesteremic effect. This serum cholesterol-lowering effect was potentiated whenever the initial level before the training program was abnormally high.

If the above hypothesis is true, what is the modus operandi underlying the hypocholesterolemic effect of exercise? This change in cholesterol concentration could be due to hormonal involvement, decreased food intake, utilization of cholesterol per se for energy or for the formation of precursors for increased steroidogenesis during periods of elevated physical activity.

The objective of this study was to determine the inter-relationship of exercise, thyroxine, and feed intake on the serum and hepatic cholesterol levels in the rat, with the possibility of elucidating the mechanism responsible for the serum cholesterol-lowering effect due to physical activity. Several modifications of methods and techniques employed by previous investigators were made in this study.

It is generally accepted that the liver is the major site of cholesterol biosynthesis, degradation, and excretion in the rat. The regulation of hepatic cholesterol metabolism is principally influenced by four physiologic variables: (i) the amount of cholesterol ingested, (ii) the caloric intake of the animal, (iii) the functional integrity of the enterohepatic circulation of bile acids, and (iv) hormones. Due to these factors, it would seem reasonable, in experimental studies,

to closely regulate the amount of ingested dietary cholesterol, bile acids, and total calories in order to insure that "normal-physiological" cholesterol metabolism occurs while the mechanism of the hypocholesteremic effects of exercise is being determined. There is little doubt that excessive dietary intake of cholesterol (Siperstein and Fagan, 1966), bile acids (Fimognari and Rodwell, 1965, Beher et al., 1959; Beher and Baker, 1959), and caloric restriction (Bucher et al., 1960; Dietschy and Wilson, 1968; Dietschy and Siperstein, 1967) will greatly affect hepatic cholesterol metabolism by suppression of cholesterologenesis. The above studies provided direct evidence that the site of negative feedback control was at the reaction responsible for the conversion of  $\beta$ -hydroxy- $\beta$ -methylglutaryl-CoA (HMG-CoA) to mevalonate. Thus, in most of the previous exercise studies, in which hypercholesterolemia was induced by dietary supplementation of cholesterol and/or bile acids to the atherosclerosis-resistant rats, caution must be employed when interpreting the effects of physical activity on the regulation of cholesterol metabolism. Other unphysiological metabolic consequences of cholesterol feeding (rabbits) for 10 to 16 weeks were reported by Forbes and associates (1964) who demonstrated adrenal hypertrophy two times the adrenal weight of the control animals; and by Bernick and Patek (1961) who showed that feeding rats one per cent cholesterol caused a marked increase in the number of thyrotropic cells in the

anterior pituitary and associated hyperplastic changes in the thyroid gland.

The importance of feeding animals a basal diet when conducting a study of this nature is also exemplified by the work of Gordon et al. (1957) who showed analogous metabolic disturbances when saturated or unsaturated fats were added to the diets of rats. The dietary fats had a significant effect on the rate of fecal elimination of bile acids and cholesterol. Under such conditions of induced hypercholesteremia, the hypocholesteremic effects of physical activity may be mitigated or even obscured. In our study the animals were fed a low-fat, low-cholesterol basal diet containing no bile acid supplementation.

Since caloric intake has been shown to have a marked effect on the rate of cholesterologenesis (Tomkins and Chaikoff, 1952; Jansen et al., 1966; Migicovsky and Wood, 1955; Hutchens et al., 1954), feed intake records were kept in this investigation. The necessity of measuring daily feed intake becomes evident when one recognizes the high correlation of physical activity with the regulation of food intake. Stevenson in 1967, reported that bouts of strenuous physical exercise caused a decreased feed intake in the rat. Mayer (1968) stated that, within a certain range of physical activity, the regulation of food intake is both precise and sensitive. Any increase in physical activity is followed by a corresponding

increase in food intake. If physical activity is below this normal range, the precision and sensitivity decreases. The regulation becomes so inaccurate that, if the animal is very inactive, the intake is actually greater than it is for slightly higher activity. Above the range of normal activity, the regulation again becomes highly imprecise and inaccurate, with the intake first increasing too slowly, then at very high levels dropping, followed by weight reduction. Thus, the possible hypocholesteremic effect of exercise could be mediated indirectly through decreased feed consumption. However, Mann and co-workers (1955c) and Karvonen et al. (1961) demonstrated that while food consumption increased (caloric intake was doubled) in active men, the level of their serum lipids did not change as long as the excess energy was dissipated as exercise or work. The diversion of excessive food calories to mechanical energy may lead to a favorable influence, such as facilitating low serum lipid levels as a consequence either of the increased muscle mass necessary to effect the energy loss or as a by-product of muscular work. This present food intake investigation should contribute greater understanding as to which of the two factors (divergence of excess calories or decreased food intake) have a major influence on the hypocholesteremic effect of physical exercise.

It has long been known that hormones, especially thyroxine and its analogues, affect cholesterol metabolism. There seems

to be little doubt of the interaction of thyroxine on hepatic cholesterol biosynthesis, degradation, and excretion. An inverse relationship between the plasma cholesterol concentration and the level of thyroid activity has long been recognized (Epstein and Lande, 1922). Although this relationship is not quantitative (Peters and Man, 1950), hyperthyroidism is usually associated with a decrease, and hypothyroidism with an increase in the serum cholesterol concentration (Kritchevsky, 1967). The mechanism by which thyroid-active compounds affect cholesterolpoiesis and cholesteroleresis has been well established. The changes in the rate of cholesterol synthesis which occur under the influence of thyroid compounds are opposite in direction from observed changes in blood cholesterol concentrations. In order for the blood cholesterol levels to vary the way they do, the rate of cholesterol destruction and excretion must dominate the synthetic rate in the hyperthyroid state and be subordinate to it in the hypothyroid state. This suggests that it is the increased rate of cholesteroleresis that accounts for the hypocholesterolemic action of thyroid-active substances. This hypothesis has been substantiated by the works of Karp and Stetten (1949), Byers and his associates (1952), Rosenman et al. (1952a, b), Eriksson (1956, 1957a, b) Boyd (1959), and Gould (1959). Since thyroid hormones are the predominant hormones that alter cholesterol metabolism, could the hypocholesterolemic effect of exercise be mediated

indirectly through the thyroid gland, producing a physiological hyperthyroid state in trained or exercised animals? This hypothesis was tested in the present study by thyroidectomizing the animals and administering graded doses of L-thyroxine in physiological amounts to produce the following thyroid states: (i) athyroidism, (ii) hyperthyroidism, (iii) hypothyroidism, (iv) euthyroidism. Rats with intact thyroids served as (v) euthyroid controls. This present method of studying the hormonal interaction of thyroxine on cholesterol metabolism becomes meaningful since past investigators have consistently used pharmacologic amounts of thyroactive substances in inducing hypocholesteremia in thyroid replacement studies. Moreover, the employment of surgical removal of the thyroid glands was employed in this investigation instead of the administration of a goitrogen, as reported in many investigations of this nature, because of the increasing reports of extrathyroidal effects induced by the goitrogen (Duncan and Best, 1958a, b; Boyd, 1959). Extensive studies have shown that such antithyroidal compounds not only block the formation of thyroxine in the thyroid gland; but also appear to alter its peripheral utilization (Flock and Bollman, 1963), including partially inhibiting deiodination of thyroxine by the extra-thyroidal tissues (Morreale de Escobar and Escobar del Rey, 1967). Also, goitrogens have been known to increase the rate of fecal elimination of L-thyroxine (Tanabe et al., 1965).

Best and Duncan (1960) concluded that goitrogens (i.e., thiouracil) have an influence on cholesterol metabolism that is not dependent upon its antithyroid effect, and it is possible that the altered cholesterol metabolism of the thiouracil-fed rat may not respond to thyroxine-like compounds in the same manner as that of the normal rat.

The effect of physical activity on its possible interaction with the thyroid gland was studied by subdividing each of the five thyroid states into four levels of physical activity: (i) no exercise, (ii) standing in three inches of water to account for the possibility of water-induced "stress", (iii) moderate exercise, and (iv) exhaustive exercise.



## REVIEW OF LITERATURE

## Cholesterol

History

In 1733, Vallinsnieri reported that gallstones were soluble in turpentine or alcohol (Kritchevsky, 1958). During the late 1960s, Poullletier de la Salle isolated the main constituent of gallstones from the alcohol extract. Conradi confirmed these findings in 1775 and also demonstrated that the constituent could be recovered from ether extract. However, it was not until 1815 that Chevreul showed that the extractable substances was unsaponifiable, which differentiated it from other waxes. The following year Chevreul gave this alcohol-ether extract its original name, "cholesterine" (Dam, 1958).

Reinitzer, in 1888, determined the exact empirical formula,  $C_{27}H_{46}O$ , and 31 years later Windaus arrived at a tentative structural formula which was changed in 1932 by Rosenheim and King to the cyclopentanoperhydrophenanthrene structure now accepted (Kritchevsky, 1958). Direct proof of the position of the hydroxyl group in the  $C_3$  position of the "A" ring was provided by Kon and Woolman (1939). Windaus and Resau isolated the side chain, methyl isohexyl ketone, that is attached to  $C_{17}$ , and Wislicenus and Moldenhauer provided evidence of a double bond in the "B" ring (Kritchevsky, 1958).

The foregoing is a brief review of the key studies on the proof of the structure of cholesterol. A more comprehensive review of this subject is given by Cook (1958), Shoppee and Shoppee (1953), Shoppee (1964), Kritchevsky (1958) and Masoro (1968).

### Biochemistry

The task of documenting all previous research on the individual steps of cholesterol biosynthesis and degradation is beyond the scope of this review. Information regarding this topic can be attained from Gould (1958), Kritchevsky (1958), Lynen (1955), Cornforth (1955), Friedman et al. (1956), Bergström and co-workers (1960), Belle (1965), and Bloch (1954, 1957). Instead, this section will be devoted only to those steps that have been proven to be part of the metabolic pathway.

Cholesterol synthesis      Page and Menschick (1932) and Schönheimer and Breusch (1933) were some of the first investigators who indicated that either biosynthesis or degradation of cholesterol could occur, depending on the cholesterol content of the diet fed, but no clear idea of the rapidity of cholesterol biosynthesis nor of the pathway was gained until tracer methods were applied to this problem. It was not until the classical work of Bloch and Rittenberg (1942) that a two-carbon unit, acetate, was clearly demonstrated to provide all the carbon atoms necessary for cholesterol biosynthesis.

Cornforth and co-workers have accomplished the remarkable feat of a systematic carbon-by-carbon dissection of the whole ring system (Cornforth et al., 1955, 1956, 1957). Although great advances have been made in establishing the enzymatic steps of cholesterol biosynthesis in the past 20 years, there are still many aspects that remain in doubt. It is certain that acetate is the principal, and probably the only, common, simple metabolite from which cholesterol is produced. Since acetyl-CoA is a major intermediate in the catabolism of fat, carbohydrate, and some of the amino acids of protein, it is evident that the animal's total cholesterol pool is derived, not only through exogenous or dietary cholesterol but, also, through the utilization of food, especially that of excessive caloric intake.

It has been unequivocally established that mevalonic acid, squalene, and lanosterol are intermediates in the biosynthesis of cholesterol. The generally accepted scheme of cholesterol biosynthesis from acetate is outlined in Figure 2. Mevalonic acid can apparently be formed from acetyl-CoA by two different enzymatic pathways: (i) two moles of acetyl-CoA form one mole of acetoacetyl-CoA plus coenzyme A; acetoacetyl-CoA reacts with another mole of acetyl-CoA plus water to form HMG-CoA. HMG-CoA is believed to be formed in the mitochondria, then moves out to the endoplasmic reticulum to form mevalonic acid when reduced by two moles of NADPH (Masoro, 1968); (ii) in an

alternate enzymatic pathway, one mole of acetyl-CoA reacts with one mole of malonyl-CoA, plus a sulfhydryl-containing enzyme, to form acetoacetyl-enzyme-complex, which reacts with one mole of acetyl-CoA and water to form HMG-enzyme-complex; the complex is then reduced to mevalonic acid by two moles of NADPH. This pathway occurs in the extramitochondrial region. It seems clearly established that both pathways can occur in mammalian tissues. The relative quantitative importance of these two pathways in the in vivo biosynthesis of mevalonic acid is not known.

Squalene was first isolated by Tsujimoto in 1906 (Gould, 1958) and was synthesized by Karrer in 1931 (Kritchevsky, 1958). By the use of dilution techniques, Langdon and Bloch (1953) showed that the rat could synthesize squalene from acetate and that this material was an exceedingly efficient precursor of cholesterol. The observation that squalene decreases the incorporation of acetate-<sup>14</sup>C into cholesterol has been advanced as evidence that squalene is an intermediate. Many confirmations of the role of squalene in cholesterol biosynthesis have since been reported (Gould, 1958).

The elucidation of the cyclization of squalene and the final steps in cholesterol biosynthesis was largely due to the investigations of Bloch and his associates. It was Voser who elucidated the structure of lanosterol in 1952 and Clayton and Bloch in 1956 who presented evidence that rat liver

synthesized lanosterol from acetate. In 1958, Gould showed that the liver rapidly converts lanosterol into cholesterol.

It is now apparent that every mammalian tissue is capable of at least some degree of de novo cholesterol synthesis. Great variation in synthetic activity among different organs, however, is apparent. In the rat (Dietschy and Wilson, 1968) and monkey (Dietschy and Siperstein, 1967) it has been demonstrated that the highest rate of sterol synthesis per unit weight of tissue occurs in two organs, liver and ileum. The lowest rates of synthesis are seen in muscle (smooth, striated, and cardiac) and mature central nervous system tissue. This difference in the potential for sterol synthesis between liver and gastrointestinal tract and the remainder of the tissues becomes even more striking when organ weight was taken into consideration and expressed as the per cent of the calculated whole body synthesis. The liver (82 per cent) and gastrointestinal tract (11.4 per cent) accounted for 97 per cent of all detectable sterol synthetic activity in the monkey (Dietschy and Siperstein, 1967). This proved that the liver is the major site of cholesterol biosynthesis.

Cholesterol degradation      Cholesterol is metabolized to bile acids, sex hormones and adrenocortical hormones. Of these, only the formation of bile acids need be considered here since it has been estimated that from 70 to 90 per cent of the cholesterol synthesized daily is converted to bile

acids in man (Ivy et al., 1957); Siperstein and Murray, 1957) and 80 to 90 per cent in the rat (Siperstein and Chaikoff, 1952; Chaikoff et al., 1952).

The existence of a direct metabolic relationship between cholesterol and bile acids was conclusively shown by Bloch and his associates (1943). The finding of Bloch and his co-workers was confirmed and extended in 1952 by Fukushima and Gallagher who studied the distribution of deuterium in the cholesterol molecule when labeled by exchange according to the method of Bloch et al. (1943). They found that the deuterium was not uniformly distributed--about 50 per cent of the label being present in the terminal isopropyl group (Fukushima and Gallagher, 1952). Since this group is lost in the biological conversion of cholesterol to cholic acid they calculated that about 85 per cent of the serum cholesterol had been converted to cholic acid. In 1952, the quantitative importance of this pathway in cholesterol metabolism in rats was established by Siperstein et al. (1952), Siperstein and Chaikoff (1952), Chaikoff et al. (1952), and Bergström and Norman (1953). They found that 80 to 90 per cent of the isotope of 4-<sup>14</sup>C-cholesterol injected intravenously into rats was excreted in the feces in 15 days. When the isotope in the stools was fractionated, 90 per cent was found to be present in the acid fraction and the rest in the neutral fraction. Cholesterol-<sup>14</sup>C introduced into the body by feeding or by intravenous or intraperitoneal

injection mixes with that in the liver and blood, and a little more slowly with that in intestine and other viscera. Its metabolic fate can therefore be considered as indicating the fate of all the cholesterol, which is in rapid equilibrium with the serum and hepatic cholesterol pool. This fate consists, to a very large extent, in oxidative removal of the terminal three carbon atoms of the side chain by the liver and eventual fecal excretion of the remaining 24 carbon atoms as bile acid metabolites. Siperstein and Chaikoff (1955) concluded that the over-all metabolism of cholesterol in rats results in the ultimate excretion of about 10 per cent as fecal sterols, 85 per cent as fecal steroid acids, and only 1 per cent in urine, presumably as steroid hormone metabolites.

Recent utilization of chromatographic methods on the isolation of bile acids has increased our knowledge of their occurrence in bile. The primary bile acids are cholic acid (80 per cent of total bile acid composition) and chenodeoxycholic acid (Bergström and Sjövall, 1954). Approximately 95 to 99 per cent of the bile acids are conjugated with taurine and the remainder with glycine. Similarly, Eriksson (1957a, b) found that the bile acids in the bile of rats are mainly cholic acid and chenodeoxycholic acids in the ratio of 8:2. The two major bile acids, differing only in a hydroxyl group at the C<sub>12</sub>, have never been shown to be interconverted from one to the other.

Two general pathways (Figure 3) in the formation of bile acids may be recognized, apparently depending on whether the side chain is cleaved before or after the C<sub>12</sub> position is hydroxylated. One of these would involve the conversion of cholesterol to lithocholic acid, which in turn would be hydroxylated at the C<sub>7</sub> position to yield chenodoxycholic acid. This compound then becomes the uncharacterized trihydroxylated bile acid. A second and quantitatively more important pathway would consist of the conversion of cholesterol to desoxycholic acid, which would then be rapidly hydroxylated in the C<sub>7</sub> position to form cholic acid as a final end-product. For further information concerning bile acid pathways, refer to Haslewood (1955), Gould and Cook (1958), Belle (1965) and Masoro (1968).

In addition to bile acids and their bacterial degradation products the feces contain other sterols, called neutral sterols, which do not contain a carboxyl group. Fecal neutral sterols are quite varied in both chemical nature and source. Some are identical to the sterols present in animal tissues (i.e., cholesterol, cholestanol, lathosterol, and 7-dehydrocholestanol). Others are products of the action of bacteria in the colon on cholesterol and other animal sterols. Of these, the most abundant is coprostanol but others such as epicoprostanol, cholestanone, and coprostanone are also present. In general, there are two sources of fecal neutral sterols, dietary and endogenous production. The endogenous



sterols (mainly cholesterol) enter the intestinal lumen in the bile secretion, in intestinal juice secretion, and from intestinal mucosal cells sloughed into the lumen. The dietary sterols are composed primarily of cholesterol and various plant sterols (i.e., sitosterol, stigmasterol, and campesterol). Since the plant sterols are poorly absorbed, almost all enter the feces as neutral sterols without having been absorbed by the mammal (Shipley et al., 1958). Man, unlike the rat, has a limited ability to absorb cholesterol (Connor et al., 1961a, b), thereby supplying greater amounts of dietary cholesterol to the feces as neutral sterol.

The final composition of the excreted bile acids is the result of an intimate interaction between the enzyme systems of the liver and those of the microorganisms in the intestine. Studies by Lindsted and Samuelsson (1959) and Olivecrona and Sjövall (1959) indicated that, in the rat, cholic acid which is secreted in the bile is partly converted into deoxycholic acid when it reaches the caecum. From this site it is incompletely absorbed and transported to the liver via the portal blood. The effective 7- $\alpha$ -hydroxylating system, which is present in the liver, hydroxylates the deoxycholic acid to cholic acid and is thus responsible for the practically complete absence of the former acid in rat bile.

On the cellular level the reaction sequence of cholesterol catabolism can be simplified to five steps occurring in the

liver: (i) hydroxylation of the nucleus of the sterol at the C<sub>10</sub> position, (ii) hydroxylation of the nucleus at the C<sub>12</sub> position, (iii) inversion of the C<sub>3</sub>-hydroxyl group, (iv) saturation of the double bond, and (v) degradation of the side chain to C-24 acid. The enzymes required for bile acid formation have been found in both the mitochondrial and microsomal regions of the liver cell (Mendelsohn and his co-workers, 1966).

Of these five steps of cholesterol catabolism occurring in the liver, the side chain cleavage to form the C-24 acid is the "committed step"<sup>1</sup> in the formation of bile acids. Suld and his associates (1962) proposed a mechanism for side chain cleavage which can be summarized as follows: (i) oxidation of a terminal methyl group to hydroxyl group, (ii) oxidation of the OH-group to a C-27 acid, (iii) oxidation at C<sub>24</sub>, and (iv) cleavage of propionic acid, leaving a C-24 acid. The enzymes involved in the side chain degradation are located in the mitochondria of rat and mouse liver cells as demonstrated by several investigators (Anfinsen and Horning, 1953; Berseus and Danielsson, 1963; Horning et al., 1957; Staple and Rabinowitz, 1962).

The rate at which cholesterol catabolism occurs depends upon the rate at which side chain cleavage occurs in the hepatic cells. There is increasing evidence that this

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<sup>1</sup>Regulation of cholesterol catabolism beyond this step becomes ineffective.

"committed step" is under the influence of hormonal regulation, namely that of thyroactive compounds (Strand, 1963; Mitropoulos and Myant, 1964). These results tend to suggest that the liver is dependent on thyroxine for effective control of the cholesterol degradation and excretion necessary to maintain normal-physiological levels of cholesterol in the organism.

Recent investigations by Malinow and his co-workers (1968a, b; 1969; 1970) on splitting of the side chain of cholesterol suggests another possible mechanism by which cholesterol catabolism occurs in the rat. They suggested that the adrenal glands also have the ability to split the terminal three carbons of cholesterol during steroidogenesis and that this is accelerated during periods of exercise. They concluded that, quantitatively, this process may be an important pathway in which exercise exerts its hypocholesteremic effect.

In conclusion, there may be two primary sites at which cholesterol degradation, in significant amounts, can occur (i) the liver and, (ii) the adrenals. The former is dependent on the level of circulating thyroxine and the latter on the rate of steroidogenesis.

#### Regulation of cholesterol metabolism

It is often stated that the level of cholesterol in the blood is a reflection of the rate of synthesis and catabolism of the sterol in the liver, dietary input, and the equilibrium with the rest of the tissues in the organism. Over the past

century there have been many factors implicated as etiologic agents affecting the serum cholesterol level.

Acknowledging the fact that cholesterol input into a system is due to endogenous synthesis and exogenous or dietary cholesterol, it was natural that the earliest studies of factors affecting serum cholesterol levels should have been concerned with the effect of dietary cholesterol. About 60 years ago, it was observed that rabbits fed diets including cholesterol had dramatic elevations of the concentrations of cholesterol in the serum and tissues. This earlier work is reviewed in the monograph of Katz and Stamler (1953). Cholesterol was, in general, fed at levels of about one gram per animal per day in the older studies and serum cholesterol concentrations rose 10 to 50 times the values seen for rabbits fed cholesterol-free diets. For some time it was thought that the rabbit was unique in its susceptibility to cholesterol-induced hypercholesteremia. Later studies have shown that, by modifying the experimental conditions, including the feeding of additional factors (i.e., bile acids, goitrogens, and saturated fat diets), hypercholesteremia could be induced in a wide range of species including the dog (Steiner and Kendall, 1946), chicken (Dauber and Katz, 1942), Cebus monkey (Mann et al., 1953), mouse (Schettler, 1949), Rhesus monkey (Cox et al., 1958), and rat (Malmros and Wigand, 1957). Man, on the other hand, has long been shown to have a poor coefficient of

cholesterol absorption when it was added to the diet, as crystalline cholesterol in oil (Keys and his associates, 1950). Recent investigations reveal that such is not the case (Connor, 1968; Connor et al., 1961a and 1961b, Barboriak et al., 1966). Since the absorption of cholesterol from the intestinal tract depends in part on the presence of fat, previous investigations on man were misleading due to the low-fat diets. Such low-fat diets, especially those of essential fatty acids, may also have had an adverse effect on the normal metabolic processes, as indicated by the discussions of Brewer and Arnrich (1958).

Certain reservations should be mentioned regarding the interpretation of past experiments on induced-hypercholesteremic rats. It has been shown in the rat, for example, that the combination of small amounts of cholesterol and cholic acid in the diet resulted in marked hypercholesteremia, whereas neither of these compounds was very active when fed alone. The interpretation of such data must be made with caution since both compounds have a direct inhibitory effect on the biosynthetic rate of hepatic cholesterol--a condition that may not be "physiological".

Not many years ago it was believed that the liver was the only site of endogenous cholesterol biosynthesis (Hotta and Chaikoff, 1955), but it seems clear now that all tissues are capable of this biosynthetic process (Dietschy and Siperstein, 1967). Although recent research by a group in Dallas

unequivocally demonstrated direct evidence for the moderate contribution by the intestinal wall to the plasma cholesterol (Wilson and Reinke, 1968; Lindsey and Wilson, 1965), the liver is still the major contributor of endogenous cholesterol (82 per cent) in the rat (Dietschy and Siperstein, 1967). Furthermore, no other organ has been shown to have the regulatory ability of the liver.

Schönheimer and Breusch (1933) was the first to show by balance studies that cholesterol metabolism in vertebrates is under the hemostatic control of the liver. Twenty years later it was conclusively proved that there is a marked compensatory suppression of cholesterol synthesis in the liver of dogs ingesting a high cholesterol diet (Taylor and Gould, 1950; Gould and Taylor, 1950; and Gould et al., 1953). Since that time, there have been voluminous reports on agents and factors associated with high and low cholesterol levels. However, under normal physiological conditions the plasma cholesterol level is under the chemostatic control of the liver--reflecting the rate of hepatic cholesterol biosynthesis versus catabolism and excretion. The regulation of cholesterol metabolism can be divided among four physiologic variables: (i) the amount of cholesterol in the diet, (ii) the caloric intake of the animal, (iii) the functional integrity of the enterohepatic circulation of bile acids, and (iv) the influence of hormones.

It should be emphasized that the conversion of HMG-CoA into mevalonic acid by HMG-CoA reductase is the "committed step" in the biosynthesis of cholesterol, since beyond this step all the processes are specific for sterol synthesis and there are no alternative metabolic pathways (Bloch, 1965).

Regulation by dietary cholesterol      Shortly after the initial reports that cholesterol synthesis could be studied in vitro in tissue slices, Taylor and Gould (1950) and Gould (1951), reported that slices of liver taken from animals on a high cholesterol intake showed marked suppression of cholesterol synthesis. This important finding was confirmed in the next two years (Tomkins et al., 1953; Gould et al., 1953; Langdon and Bloch, 1953; and Frantz et al., 1954). The enzymatic site most responsible for this diminished activity has been shown to be early in the biosynthetic pathway. Gould and Popják (1957) provided evidence that this reaction site is located prior to mevalonic acid (MVA).

This phenomenon was investigated further by Siperstein and Guest (1960). They demonstrated that liver slices taken from animals fed a high cholesterol diet could incorporate acetate-<sup>14</sup>C into carbon dioxide, fatty acids and ketone bodies at near control rates even though cholesterol synthesis in these slices was almost completely suppressed. Therefore, the point of inhibition could be identified, indirectly, as the reduction of HMG-CoA to MVA.

In 1966, Siperstein and Fagan provided direct evidence of the site of negative feedback control of cholesterol biosynthesis. Assay was made by direct measurement of the various intermediate compounds in cholesterol synthesis with the use of gas-liquid chromatographic procedures, both in a cell-free system of liver and in intact liver cells of rats. Mevalonate synthesis was markedly suppressed by cholesterol feeding, while the synthesis of HMG-CoA was unaffected. The conclusion was that the major site of the cholesterol feedback system was located at the reaction of HMG-CoA to MVA, namely HMG-CoA reductase.

Although the reduction of HMG-CoA reductase activity is the primary site of feedback control of cholesterol synthesis, after prolonged cholesterol feeding diminished enzymatic activity appears at other steps in the biosynthetic sequence. Siperstein and Guest (1960) for example, reported that after rats were fed cholesterol for 11 days the rate of conversion of mevalonate- $^{14}\text{C}$  to cholesterol by liver slices decreased to 35 per cent of control values at a time when the rate of acetate- $^{14}\text{C}$  incorporation into cholesterol was suppressed to five per cent of control levels. Thus, whereas the rate-limiting step was still the reduction of HMG-CoA, marked reduction of enzymatic activity after mevalonate formation was clearly demonstrable.



More recently, Gould and Swyryd (1966) have shown that after prolonged cholesterol feeding, there was considerable reduction in the ability of liver homogenates to convert mevalonate to farnesyl pyrophosphate and farnesyl pyrophosphate to squalene. This depression of enzymatic activity is almost certainly secondary to primary feedback inhibition of HMG-CoA reductase during cholesterol feeding and probably has no direct role as a control mechanism of overall sterol synthesis by the liver in the intact animal.

Dietschy and Siperstein (1967) fed rats a five per cent cholesterol diet and found that virtually complete suppression of hepatic cholesterogenesis occurred. Moreover, feeding a high cholesterol diet for as short a period as 12 hours caused considerable depression of hepatic sterol synthesis, and suppression was nearly complete within 48 hours. Thus, cholesterol feeding produces both a prompt and a marked suppression of cholesterol synthesis by liver. No other tissue besides the liver shows such marked suppression of sterol synthetic activity (Dietschy and Siperstein, 1967).

Finally, the time sequence for recovery of hepatic cholesterogenic activity after cessation of cholesterol intake is related to the duration of cholesterol feeding. Taylor and his associates (1956), for example, have shown that the rate of hepatic sterol synthesis returned to normal after one day of cholesterol deprivation if the cholesterol diet had been

given for only 24 hours; in contrast, if animals were maintained on a high cholesterol intake for two months, 15 days were required for restoration of normal sterologenic activity in the liver.

Regulation by caloric intake      In 1952, Tomkins and Chaikoff demonstrated that fasting as well as cholesterol feeding brings about a marked reduction in cholesterol synthesis by the liver. This finding was subsequently confirmed in other laboratories (Migicovsky and Wood, 1955; Bucher et al., 1959; Sauer, 1960; Jansen et al., 1966; Dietschy and Siperstein, 1967; and Dietschy and Wilson, 1968). It is evident from the study of Dietschy and Siperstein that the most striking effect of this dietary manipulation is upon cholesterol synthesis by the liver. In these particular experiments fasting for 48 hours reduced hepatic cholesterogenesis in the rat by 11 times. In all seventeen tissues studied, including the various levels of the gastrointestinal tract, there was little or no reduction in the rate of cholesterol synthesis as measured by in vitro techniques. Scaife and Migicovsky (1957) found indications of a metabolic block between HMG-CoA and squalene in liver homogenates from fasting animals. Bucher and his co-workers (1959 and 1960) later provided evidence that the activity of HMG-CoA reductase was decreased in the liver of fasted rats and that there was also a partial block between squalene and cholesterol.

Although no studies have been reported on the effects of excess calories on HMG-CoA reductase activity, there is little question that the substrate, acetyl-CoA, can be derived from immoderate intake of calories. There seems to be little doubt that excessive total calories can cause hypercholesterolemia (Keys, 1952; Keys et al., 1950; Moses, 1952; Mann et al., 1955a, b). Controlled studies on human subjects (Mann et al., 1955c; Calvy et al., 1963, 1964) show that man is able to consume high-fat diets and double their caloric intake without increasing the level of their serum lipids so long as the excess energy is dissipated as exercise. Albrink (1965) suggested that the metabolic consequences of caloric excess, rather than any one particular kind of food, are responsible for the increase in atherosclerosis. A study by Walker et al. (1953) disclosed that strong positive caloric balance in two subjects on a very low lipid intake was associated with significant increases of the serum cholesterol and lipoproteins. This led to the conclusion that caloric balance appears to play a major role in controlling serum lipid levels. If fasting can reduce hepatic HMG-CoA reductase activity, it would seem probable that the excess substrate, acetyl-CoA (which is the precursor for cholesterol biosynthesis), derived from caloric excess could lead to increase in HMG-CoA reductase activity in a direct or indirect manner.

Bloomfield (1963) demonstrated that cholesterol synthesis was caloric-dependent, in which caloric intake provided the necessary energy for the process. It has been shown that cholesterolgenesis is dependent on the availability of ATP and NADP (Fletcher and Myant, 1960). Thus, it is possible to conclude that caloric intake may affect cholesterol metabolism either directly through HMG-CoA reductase activity or by another rate-limiting step, the availability of the co-factors ATP and NADP.

#### Regulation by the enterohepatic circulation of bile acids

It is doubtful whether bile salts occur in sufficient concentration in the human or animal diet to be of practical importance in the regulation of serum cholesterol levels. The bile acids merit consideration in this review because of their importance in the production of experimental hypercholesteremia and because the metabolism of endogenous bile acids affects cholesterol metabolism. Page and Brown (1952) were among the first investigators to report the use of dietary bile acids in systematic studies of hypercholesteremia in rats. The combined use of  $^{131}\text{I}$ -induced hypothyroidism with diets containing two per cent cholic acid and four per cent cholesterol produced very high levels of serum cholesterol and massive cholesterol infiltration of tissues. Liver cholesterol concentrations of over 14 per cent, on a wet-weight basis, were observed. Hegsted and associates (1957) evaluated, for their hypercholesteremic potency, several dietary levels of cholic acid

fed with various levels of cholesterol. Cholic acid at 0.15 per cent of the diet produced a definite effect, and a linear response was obtained with log concentrations of cholic acid as high as 1.35 per cent. The degree of inhibition was proportional to the serum cholate level (Beher and Baker, 1959). Conversely, any experimental manipulation that depleted the enterohepatic circulation of bile acids led to an enhanced rate of cholesterol synthesis in the liver. Such depletion may be accomplished by external diversion of the bile, as has been reported in many species including the rat (Weis and Dietschy, 1969; Myant and Eder, 1961; and Danielsson *et al.*, 1967), by administration of the bile acid sequestrant cholestyramine (Huff and co-workers, 1963), or by ileal bypass (Moutafis and Myant, 1968). The mechanism of inhibition of hepatic cholesterol biosyntheses due to bile acids is similar to that of excessive cholesterol intake or by fasting namely, the inhibition of HMG-CoA reductase activity in the liver. This was demonstrated by Fimognari and Rodwell (1965).

Regulation by hormones      It has long been known, both from clinical observations and from experiments on laboratory animals, that the metabolism of lipids is influenced by hormones, particularly by thyroxine. Among the lipids, cholesterol shows the greatest regularity in response to the circulating level of thyroid hormone but other lipid components are affected similarly (Pitt-Rivers and Tata, 1959).

According to Mason et al. (1930), Magnus-Levy made the fundamental observation in 1895 on the influence of the thyroid on endogenous adiposity. He also noted that in all cases of myxedema and in many cases of endogenous adiposity, thyroid insufficiency was noted.

In 1914, Martinez (1917) of South America reported that he had found a decrease in blood cholesterol within the first 24 hours after thyroidectomy (dogs) followed by a progressive increase thereafter.

In the United States Epstein and Lande (1922) called attention to an inverse relationship between blood cholesterol values and metabolic rates. Gardner and Gainsborough (1928) obtained results similar to Epstein's but refrained from attaching great significance to them because of the many exceptions to the findings.

Baumann and Holly (1926) reported that thyroidectomy of rabbits resulted in a rise in serum cholesterol. In spite of this increase, the hypercholesteremic effect was lost when the rabbits became pregnant.

Wade (1929) reported slightly increased values of blood cholesterol in patients with toxic goiter and found even higher values after total thyroidectomy.

Mason et al. (1930) and Hurxthal (1933a, b; 1934a, b) consistently found hypercholesterolemia in myxedemic patients, and a less regular but significantly lower blood cholesterol

in thyrotoxicosis. Their exhaustive study on over 500 patients led to the following conclusions: (i) postoperative myxedema is accompanied by hypercholesteremia; (ii) subtotal thyroidectomy may be followed by hypercholesteremia without clinical myxedema, which is interpreted as a transient thyroid deficiency; (iii) subtotal thyroidectomy may be followed by low metabolic rates without hypercholesteremia (myxedema was seldom found in these cases); (iv) thyroid deficiency produces myxedema and hypercholesteremia, but at times myxedema may be clinically imperceptible; (v) hypercholesteremia, when not explainable on any other basis, may be considered as possibly of thyroid origin and is a rational indication for thyroid administration; (vi) the finding of hypercholesteremia, in the absence of its few other common causes, points more specifically to thyroid deficiency than does the finding of a low metabolic rate; (vii) the relationship between the blood cholesterol and the basal metabolism is usually reciprocal when they undergo change as the result of variations in the activity of the thyroid gland or thyroid compounds in the body; and (viii) blood cholesterol provides another variable which may be used as a guide in the treatment of thyroid disease.

Goldbloom and Gottlieb, in 1927, and Bronstein, in 1933, found an increase of blood cholesterol in cretin subjects. Similar results in cretin rabbits were found; furthermore, feeding desiccated thyroid caused a reduction in serum

cholesterol--a condition that was difficult to duplicate in normal rabbits (Westra and Kunde, 1933).

Observing hypercholesteremia in patients with low metabolism and no clinical evidence of hypothyroidism, Gilligan and collaborators (1934) reported that many patients failed to keep a consistent relationship between basal metabolism and blood cholesterol level after thyroidectomy. That same year, experimental investigations of Cutting and his co-workers (1934), as well as that of Grant and Schube (1934), supported that belief. They obtained no correlation whatever between blood cholesterol and metabolic elevations induced by dinitrophenol. Thus, a more valid correlation is the relationship of thyroid activity and cholesterol level. Abundant data have since supported that principle, illustrating hypercholesteremia in hypothyroidism (Kunde et al., 1932; McGee, 1935; Boyd, 1936; Boyd and Connell, 1936; Turner et al., 1938; Schmidt and Huges, 1938; Gildea et al., 1939; Kendall, 1939; Fleischmann et al., 1940; Lyons and Cashman, 1941; Chaikoff et al., 1941; Fleischmann and Wilkins, 1941; Fleischmann and Shumacher, 1942; Entenman et al., 1942; Peters and Man, 1943; Forbes, 1944; Fleischmann and Fried, 1945; Foldes and Murphy, 1946; Steiner and Kendall, 1946; Chanutin et al., 1947; Horlick and Havel, 1948; Handler, 1948; Peters and Man, 1950; Blumgart et al., 1950; Stamler et al., 1950; Marx et al., 1950; Page and Brown, 1952; Rosenman et al., 1952a, b; Weiss and Marx, 1955; Byers,



1958; Deming et al., 1958; Duncan and Best, 1958a, b; Best and Duncan, 1959; Thomas and Hartroft, 1959; Gould, 1959; Boyd, 1959; Oliver and Boyd, 1959; Kritchevsky, 1960; Kritchevsky et al., 1960; Boyd, 1961; Kurland et al., 1961; Wells and Ershoff, 1962; Ellefson and Mason, 1962; Patek et al., 1963; Myant, 1964; Lepp et al., 1964; Tsung-chin and Shih-chen, 1965; O'Hara et al., 1966; Miettinen, 1968; and Furman, 1969).

Similarly, evidences for the relationship between hypocholesteremia and hyperthyroidism is well documented (Kunde et al., 1932; Turner, 1933; Turner et al., 1938; Schmidt and Huges, 1938; Fleischmann et al., 1940; Fleischmann and Shumacher, 1942; Entenman et al., 1942; Fleischmann and Fried, 1945; Chanutin et al., 1947; Dauber et al., 1949; Stamler et al., 1950; Marx et al., 1950; Rosenman et al., 1952a, b; Marx et al., 1953; Byers, 1958; Duncan and Best, 1958a; Gould, 1959; Boyd, 1959; Boyd and Oliver, 1960a, b; Cuthbertson et al., 1960; Best and Duncan, 1960; Kritchevsky, 1960; Kritchevsky et al., 1960; Boyd, 1961; Kritchevsky et al., 1961; Greene et al., 1961; Wells and Ershoff, 1962; Ellefson and Mason, 1962; Davis et al., 1962; Jepson, 1963; Patek et al., 1963; Myant, 1964; Felt, 1966; Kritchevsky and Tepper, 1967; Miettinen, 1968; and Young, 1968).

The correlation on the relationship of thyroid status and serum cholesterol concentration became more meaningful when the studies were directed to its relationship on the cellular level. It was not until 1949 that the study of Karp

and Stetten suggested that thyroxine had a direct effect on cholesterol metabolism. The turnover of the plasma cholesterol in vivo was studied in intact and thyroidectomized rats using acetate as a precursor. Assuming that the endogenous cholesterol of the plasma, both in its free and esterified forms, is synthesized mainly in the liver (Friedman et al., 1951; Gould, 1951; Friedman and Byers, 1955; Dietschy and Wilson, 1968), a change in the rate of replacement of the plasma cholesterol should, therefore, be reflected as a change in the rate of hepatic cholesterol biosynthesis. Their data revealed that the turnover of plasma cholesterol in thiouracil-treated rats was small but significantly decreased when compared to the controls. Using deuterium as a marker, they also showed an increased incorporation of isotope into sterol in hyperthyroid rats. Byers and co-workers (1952) used tritiated water and found that in hyperthyroid rats the rates of cholesterol synthesis and turnover were greater than in normal rats, while in hypothyroid rats both synthesis and turnover rate were lower. These findings on the direct correlation of the amount of circulating thyroxine or its analogues to cholesterol cholesterolgenesis turnover rates have been confirmed using labeled acetate (Dayton et al., 1960; Tsung-chin and Shih-chen, 1965; Eskelson et al., 1970), deuterium (Marx and co-workers, 1953), and cholesterol-<sup>14</sup>C (Gould, 1959).

In experiments using liver slices, Fletcher and Myant (1958) disclosed that thyroidectomized rats had lowered rates of biosynthesis of cholesterol from acetate by 80 per cent, while thyroxine injection caused a 2-3 fold increase in synthesis rate. There was no effect on the rate of biosynthesis from mevalonic acid. Thus, the biosynthetic step being affected would appear to lie between acetate and mevalonate. Similar results were obtained the following year by Boyd (1959).

Recently it has been found that in thyroidectomized animals a decrease in the level of HMG-CoA reductase occurs. The administration of thyroxine increases the levels of this enzyme resulting in increased cholesterolgenesis (Gruder et al., 1968 and Gries and co-workers, 1962).

The changes in the rate of cholesterol synthesis which occur under the influence of thyroid-active materials are opposite in direction from the observed changes in serum cholesterol concentrations. In order for the blood cholesterol levels to vary the way they do, the rate of cholesterol catabolism and excretion must dominate the synthetic rate in the hyperthyroid state and be subordinate to it in the hypothyroid state.

The extensive studies of Rosenman and co-workers (1952a, b) revealed that there was a decreased output of biliary cholesterol in the hypothyroid rat and an increase in the hyperthyroid rat. Moreover, the rates of catabolism, as

demonstrated by half-life ( $t_{\frac{1}{2}}$ ) of the labeled cholesterol, were 17.3 days for the controls, 43.0 days for hypothyroid and 7.3 days for the hyperthyroid rats. This was confirmed by Thompson and Vars (1953a, b, and 1954), who, in addition, found a decrease in the excretion of cholic acid both in hyper- and hypothyroid states. However, chenodeoxycholic acid was not mentioned, which is, quantitatively, the second most important bile acid in the rat (Bergström and Sjövall, 1954). Eriksson (1957a, b), in a detailed study of the biliary excretion of both cholic and chenodeoxycholic acid in eu-, hyper- and hypothyroid bile-fistulated rats, found a decreased excretion of cholate in both hyper- and hypothyroidism. Also, there was a substantial increase in the amount of chenodeoxycholic acid in hyperthyroidism so that the total excretion of bile acids was at least the same as in normal. In hypothyroidism the amount of chenodeoxycholic acid excreted was less than in the euthyroid state. These results suggest the possibility of a direct inhibitory effect of thyroxine on the 12 alpha-hydroxylase or a stimulatory one on the side-chain oxidation, as there was almost a reversal of the normal ratio of cholate to chenodeoxycholate of 4:1 in the hyperthyroid state. Similar results were attained by Zyl (1957), Strand (1963), Lin et al. (1963), Lepp and co-workers (1964) and Miettinen (1968), but not by Gans (1960). The findings of Strand (1962) denoted that, although the effects of thyroid activity on the

excretion of bile acids were the same as those observed by Eriksson (1957a, b), the proportionate change of the two major bile acids differed. It was shown that D-T<sub>3</sub> administration caused a change in the normal taurochenodeoxycholate/taurocholate ratio, 1:3 to 3:1. In the propylthiouracil-treated rats, total daily production of bile acids was only 15-20 per cent of that found in normal and thyroid hormone-treated rats, and the bile acid ratio was 1:9.

Referring to earlier studies by Rosenman et al. (1952b), the hyperthyroid rat cleared intravenously-injected cholesterol at a faster rate than did normal or hypothyroid rats. The hypercholesteremic rat plasma injected into normal, hypo-, and hyperthyroid rats resulted in 71.2, 59.7, and 95.6 per cent excretion respectively, of the exogenous cholesterol six hours after administration. Weiss and Marx (1955) injected cholesterol-4-<sup>14</sup>C into hyper- and hypothyroid rats and found that the conversion of cholesterol to acidic products, as well as excretion of both acidic products and neutral sterol, was stimulated by thyroid hormones. Calculations of bile acid synthesis and pools in the intact rat indicated that when D- and L-T<sub>3</sub> are administered the total biliary acid synthesis increases as a result of increased chenodeoxycholate synthesis (Strand, 1963) without alteration in rate of synthesis of cholate. The results of the following experiments can be interpreted to indicate that the thyroid hormone can stimulate

the degradation or modification of cholesterol, a prerequisite step for the formation of bile acids. The mechanism by which this occurs is not clearly understood. It has been shown that liver mitochondria can oxidize the end carbon atoms of the cholesterol side chain to  $\text{CO}_2$  (Anfinsen and Horning, 1953) with the production of substances closely related to naturally occurring bile acids (Fredrickson, 1956). In vitro studies on liver mitochondria from thyroxine-treated rats demonstrated that four times as much  $^{14}\text{CO}_2$  was produced in the treated rats as compared to mitochondria from untreated rats (Mitropoulos and Myant, 1964). This opens the possibility of a mechanism by which the rate of cholesterol degradation influenced by thyroxine. Thyroxine may stimulate a rate-limiting reaction at one of the later steps concerned in the modification of the side chain of cholesterol. This step could be the cleavage reaction resulting in the formation of a C-24 bile acid and propionyl-CoA (Suld et al., 1962). It was also suggested that thyroxine could stimulate the enzyme catalyzing the formation of di- and trihydroxycoprostanic acids from their respective precursors, di- and trihydroxycoprostanane. This could explain why thyroid-active compounds stimulate the production of chenodeoxycholate to a greater extent than that of cholate, since 3, 7 alpha-dihydroxycoprostanane is not only the precursor of chenodeoxycholate, but is also the immediate precursor of 3,7,12 alpha-trihydroxycoprostanane (an intermediate in the

formation of cholate}. However, Kritchevsky and his associates (1962 and 1965) were not able to demonstrate the dependence of cholesterol oxidation in vitro on thyroxine. Despite this discrepancy on the mechanism by which thyroxine influences the conversion of cholesterol into bile acids, there is little doubt that the increased rate of disappearance of cholesterol by catabolism and excretion (to bile acids) accounts for the hypocholesterolemic action of thyroid active substances.

### Physical Activity

Dawson was one of the first authorities to suggest exercise as a therapeutic agent as well as a preventative measure for circulatory diseases. His phonetic description is given verbatim both as evidence and for the appreciation of his writing style:

Patients with arterial sclerosis wer placed upon a see-saw & gradually tipt up and down. Their symptoms, confusion & hedake, & their objective signs, color & mental condition, wer improved. Thus, one is led to ask, Mite not these gentle oscillations be duplicated in lite calisthenics & mite not the latter tend to prevent as wel as alleviate the condition? (Dawson, 1935, p. 890)

Physical conditioning and physical fitness have been given copious attention by the American public in recent years. Many of our nation's leaders, notably the late President John F. Kennedy, have expressed their interest in and support of active physical fitness programs. The President's Council on Physical Fitness indicates continuing high-level interest in

this important subject.

Research interests have been stimulated by the recognition of the association of the incidences of obesity, hyperlipaemia, and coronary heart disease to the lack of physical activity. Volume of research in this area in the past two decades is overwhelming. For the sake of simplicity, this review will be divided into epidemiological, human experimental, and animal experimental findings.

### Epidemiological research

Although there are abundant population studies demonstrating the correlation of incidences of angina pectoris, development of myocardial infarctions, or deaths from coronary heart disease to physical activity or inactivity, for the sake of brevity, this section will be primarily limited to the association between cholesterol level and physical activity. A review of the former aspects can be attained from Heyden (1969), Fox and Haskell (1968), Fox and Skinner (1964), Holloszy (1963), and Hein and Ryan (1960).

While the literature regarding the effect of exercise on decreased incidences of myocardial infarction and heart disease seems quite coherent (Kannel and McNamara, 1967; Morris and Crawford, 1958; Stamler et al., 1960; Breslow and Buell, 1960; and Kahn, 1963), its relationship to serum cholesterol levels seems particularly confusing.



Positive correlations      Early in 1955, a report on the serum lipoproteins and cholesterol concentrations of Central and North Americans by Mann and co-workers (1955a) made the first inference regarding cholesterol levels associated with physical activity. Total cholesterol and beta-lipoprotein measurements of a group of rural Central American subjects subsisting on a largely vegetarian and low-fat diet were compared with those of urban Guatemalan and North American subjects who habitually consumed large amounts of fat (36 and 40 per cent of the total calories coming from fat, respectively). The study revealed an unexpected dissociation of the serum beta-lipoprotein and cholesterol levels. The rural Central Americans showed low and almost constant cholesterol levels at all adult ages. The levels, which were near 150 mg per 100 ml for both sexes of that adult population, are characteristic of North American children (Lee, 1967). In contrast, the serum beta-lipoprotein levels in the Central Americans were similar to those of the North American subjects when compared according to age and sex. The differences in dietary fat intake among the groups could not be used to explain the serum lipid differences because of the many reports that have shown the reduction of both the serum cholesterol and beta-lipoprotein levels when dietary fat intake was limited (Gofman et al., 1954; Lyon et al., 1956). The only other difference was that the studies in Central America indicated that, although the subjects were thinner than the age- and sex-matched North

Americans, the Central Americans consumed more food daily in relation to body size. These facts were interpreted to indicate that the high energy expenditures by the Central Americans must have accounted for the weight differences--a reasonable expectation in an agrarian culture. This study indicated that a salutary effect of muscular exercise in regulating serum lipids might be possible.

Similar findings on 46 Nigerian men who had larger muscle mass and magnitude of energy expenditure compared to an age- and weight-matched group of U.S. citizens, supported the earlier finding (Mann et al., 1955b).

A study was conducted in Africa on serum cholesterol levels of normal Bantu men that were all migrant laborers, and normal white men (controls) that were mainly white collar workers from a large Cape Town insurance company. The study showed a serum total cholesterol average of 147 mg per 100 ml for the former group and 243 mg per 100 ml for the latter. Total daily caloric intake was relatively similar between both groups (Merskey et al., 1960). It was concluded that physical activity accounted for the difference in serum cholesterol levels.

Mathur (1960) investigated various factors related to coronary heart disease (CHD) in two groups of patients: (i) 553 patients with clinical CHD and (ii) 1,056 persons selected in a field survey of the general population of Agra, India. The incidence of CHD was high in the urban population,

in the upper socioeconomic classes, and in persons with the highest amount of total dietary fat consumption. Physical activity was found to be less, and emotional stress and strains were greater, in patients with CHD. Business executives formed the largest percentage of the 553 patients with CHD, with doctors, landlords, and high government officials following in that order. In evaluating the amount of physical activity involved in each case study, it was calculated that the higher the strata of the society the higher the serum cholesterol concentration. This was in marked contrast to the poorer sections of society where type of work and mode of life demanded a greater amount of physical activity.

In 1961, Karvonen and his associates studied the food consumption of lumberjacks in five camps in eastern Finland. These men had an extremely large average daily food intake, over 4,700 kcal, of which no less than 45 per cent were derived from fat. Yet their serum cholesterol levels were no higher than those of the average Finnish man with a much lower caloric intake, only 35 per cent of which is derived from fat.

The Samburu people of Northern Kenya live on a diet of milk and meat. Sharper et al. (1961, 1962) found that all the males (15-60 years old) had very low serum cholesterol levels (mean = 166 mg per 100 ml) despite the moderate to high saturated fat intake. It is most interesting to note that at all age levels there is a high degree of physical activity.

Their activity is associated with herding, watering, and grazing of cattle. Family migrations usually take place every six weeks; the whole settlement moves 10-15 miles, whereas, seasonally, cattle may be driven by the younger men to areas 50 or even 100 miles distant in search of grazing land or water. The task of herding the main stock is undertaken by older boys, warriors, and elders. A day's herding may involve a trek of up to 20 miles. Warriors and elders who are not herding will assist with watering cattle and this may involve lifting 400 gallons of water four to five feet away by hand every other day.

Gsell and Mayer (1962) compared an extremely active Swiss village population with an urban Swiss population. The village population had a high-fat intake and a daily caloric intake 1,000 kcal higher than the urban population. However, the serum cholesterol levels of both men and women were significantly lower than those found in their urbanized countrymen.

Hard physical labor as a way of life of the West Indian Community (St. Kitts, West Indies), revealed a similar inverse relationship with serum cholesterol (Stuart et al., 1962). Average serum cholesterol level, for ages 20 to 49, was 177 mg per 100 ml, which is considerably lower than the North American's (230 mg per 100 ml) in a similar age-matched group (Schilling et al., 1964).

A six-year cohort and descriptive epidemiologic study of the Many Farms Navajo Indian population has revealed only four cases of CHD in 508 adults aged 30 years and older (Fulmer and Roberts, 1964). The incidence of CHD was significantly lower when an appropriate age- and sex-matched segment was compared to the Farmingham population (Kannel and McNamara, 1967). The dietary survey indicated that the Navajo had a high animal fat intake of adequate caloric value, but cholesterol levels appeared to be somewhat lower than in the Framingham group. The Navajo occupational activity, for the most part, involves moderate to heavy labor, with much walking and horseback riding. It was estimated that the Navajo compares with other North American rural groups in terms of physical activity, which was considerably greater than that found in a white urban population.

A study was carried out on 26 pairs of white men matched age for age and classified on the basis of high or low serum cholesterol value; the nutrient intake was accessed by means of dietary interview, and exercise was evaluated on the basis of occupation. A significant correlation was not found between serum cholesterol and the dietary components. While a highly significant inverse relationship was found between exercise and serum cholesterol level (Stulb et al., 1965).

In 1965, Melichar examined the serum cholesterol of three groups: (i) 67 individuals who had been working for 5 to 10

years in the foundry, all performing hard physical work; (ii) 47 administrative employees who had also been working for 5 to 10 years, but who had, in many cases, some regular form of physical activity; and (iii) 56 civil servants who expended great mental exertion and who had no spare time for regular physical exercise. In spite of the fact that the highest average and highest consumption of fats (consisting almost exclusively of lard and butter) were found in the group doing hard physical work (group one), the serum total and esterified cholesterol were the lowest. The lipid values of the second and third groups did not differ significantly from group one. However, in the second and third groups the number of overweight persons was higher. When the serum cholesterol was evaluated on those who were slightly underweight in the latter two groups, a positive relationship occurred between the cholesterol concentration and the lack of activity.

If excess weight can be used as an indirect index of inactivity, then other investigations also demonstrated a positive relationship between inactivity and high serum cholesterol level (Gofman and Jones, 1952; Walker, 1953; Lewis et al., 1957; Lawry et al., 1957; Thomas and Garn, 1960; Hunter and Wong, 1961; Stamler et al., 1962).

Negative correlations      An extensive survey by Lewis et al. (1957) on total serum cholesterol levels of 10,690 men and 3,404 women of the normal population varying in occupations

(skilled, semi-skilled, and unskilled workers including prisoners) revealed no relationship between physical activity and the varying cholesterol levels.

In 1956, Keys and his collaborators (1956a) presented results of a lengthy epidemiological study on serum cholesterol of men classified by age and physical activity in Minnesota, Malmö, Sweden, Bologna, Naples, the Islands of Sardinia and three ethnic groups in Cape Province, South Africa. It was concluded that physical activity did not explain the large differences in serum cholesterol when groups with different dietary habits were compared.

Sharper and Jones (1959) found no correlation between physical activity and serum cholesterol levels of young Africans and Asians, aged 12 to 20.

An epidemiologic survey of the incidence of myocardial infarction in 8,500 middle-aged members (40 to 65 years old) of Israeli Kibbutzim during a 10-year period showed that the ratio of incidence of myocardial infarction in sedentary male workers to members engaged in manual work was 3:1 (Brunner et al., 1962a). In contrast, the average values of serum total cholesterol showed a tendency to be lower in the various age groups of sedentary workers than in the members engaged in heavy or light manual work. According to the constitution of the cooperative settlements, all members live in absolute equality regarding their nutrition, housing conditions, and all

other environmental factors irrespective of the nature of work or status. In this study the implication of the results of the survey may have a more definite meaning due to the more controlled environmental conditions.

It was reported by Lee et al. (1962) that serum cholesterol levels of young (mean = 21.3 years old) and older (mean = 41.2 years old) Buddhist monks and nuns were 119 and 122 mg per 100 ml respectively. Although these Korean monks and nuns are strict vegetarians, consuming an exceedingly low fat diet (7 per cent of total calories), they live a very sedentary life. On the other hand, his comparison on a more active group, U.S. soldiers (average age, 21.6 years old) and officers (mean = 43.3 years old) had serum cholesterol levels of 192 and 231 mg per 100 ml respectively. Therefore, they concluded that physical activity did not seem to be the hypocholesteremic factor when the non-active monks and nuns were compared to the active soldiers and officers.

Emotionally excitable persons and those with completely sedentary living habits displayed no relationship between personality patterns or exercise habits to serum cholesterol levels (Raab and Krzywanek, 1965).

In 1966, Cooper et al. reported serum cholesterol values of 30 veteran handball players, age 30 to 69. They found no significant lowering of serum cholesterol in this active group (mean = 254 mg per 100 ml).



Taylor et al. (1967) reported that serum cholesterol levels of three groups of U.S. railroad employees (non-sedentary clerks, sedentary clerks, and switchmen) bore no significant relationship to the activity levels in each of the age groups 40-44, 45-49, 50-54, and 55-59.

Similarly, Malhotra (1967) reported serum lipid concentrations in 28 pairs of age-matched railway men from two geographically different Indian population groups (Udaipur and Madras) with disparate consumption of fats. A marked difference existed between the two groups with respect to mortality rate from ischemic heart disease. His data showed no significant differences in total and esterified cholesterol, even though one group (blood donors from the railway hospital in Madras) was less active than the physically active sweepers.

#### Human experimental research

Numerous human experimental studies have occurred since the initial epidimological findings in the early 1950s on the possible relationship of exercise to serum lipid levels.

Positive correlations Mann et al. in 1955c, reported the effects of vigorous physical exercise on the serum cholesterol levels of four medical students. Their observations indicated that the men were able to consume high-fat diets and double their caloric intake without increasing the level of their serum lipids so long as the excess energy was dissipated as exercise. A similar finding was reported by Calvy

and co-workers in 1963 on 101 Marine trainees (average age, 20.5) on a 4,500 kcal diet (10 per cent protein, 45 per cent fat, and 45 per cent carbohydrate). The men followed a strenuous regimen of exercise, even greater than that of the lumberjacks (Karvonen et al., 1961), from the moment of arising at 5:00 am until they went to bed at 9:30 pm. Although the caloric intake was excessive, the 22 weeks of training inhibited the rise of serum cholesterol. Calvy et al. (1964) confirmed these findings.

A comparison was made with samples from male students of the University of Minnesota, 100 of whom were studied in the basal, fasting state while 300 others came in between classes several hours after a normal breakfast. In both groups in which the men were relatively inactive after breakfast, the serum total cholesterol tended to rise. However, in another group in which breakfast was followed by moderately vigorous physical work (walking on the treadmill or working in the garden), the post-breakfast rise did not occur; instead, there was a decrease. In another segment of the investigation, cholesterol was added to the meal to examine the effect of exercise on serum cholesterol levels. Serum cholesterol concentration increased approximately five-fold in the cholesterol-fed group that did no exercise after the meal. The cholesterol-fed group that did exercise after the meal (walked on the treadmill for 45 minutes out of every 60 minutes up to 7 hours) had a serum total concentration higher than the basal

diet group but lower (45 per cent) than the cholesterol-fed group that had no post-breakfast exercise (Keys et al., 1956b).

The effect of increasing the level of daily physical activity on serum cholesterol concentration was studied in nine clinically healthy university students (Taylor and co-workers, 1957). Physical activity was induced by walking on a motor-driven treadmill at a rate which required 1,280 kcal for two hours. The caloric intake was increased by 900 kcal but the proportion of calories derived from fat was held constant. There was no significant change in the serum cholesterol level. It was concluded that the serum cholesterol concentration is determined by the fat transport load from the intestine to the liver and to the fat depots which in turn is influenced by the circulation rate. Therefore, it is related to the proportion of total calories derived from fat.

Five sets of experiments by Taylor et al. (1961) were performed on young males. Each set consisted of a sedentary control period of two weeks which was followed by a week of conditioning on the treadmill and succeeded by two weeks of moderate exercise (subjects expended 1,200 kcal per day walking 2 hours at 3.5 miles per hour on a 10 per cent grade). The final period of sedentary activity lasted 2 weeks. In each set of experiments the total caloric intake was adjusted to maintain body weight ( $\pm$  2 lb). The per cent of calories from fat (principally saturated) during the five exercise periods was 43, 53, 32, 20, and 55 which produced the following changes

in serum cholesterol concentration at the end of the exercise period: 1.7, -18.4, -22.5, +3.0 and 1.5 mg per 100 ml. In the last experiment, the recovery diet was maintained at 55 per cent of the calories from fat and the serum cholesterol concentration increased 15 mg per 100 ml in 2 weeks. It was concluded that when saturated fat predominates, the serum cholesterol concentration during moderate work is controlled by the per cent fat in the diet up to approximately 40 per cent of total calories. Thereafter, a moderate physical work load results in a lower serum cholesterol than expected.

In 1961, Golding conducted a study to determine the effects of a hard endurance exercise program on total serum cholesterol in young college men. Serum cholesterol values were determined before and after the program on four male subjects and four controls. The exercised group participated in a 25-week training period in which strength and endurance were stressed. The subjects met for one hour of exercise five days a week and were encouraged to participate in physical recreation activity on the weekends. All four exercised subjects showed significant reduction in serum cholesterol at the end of the 25-week period. Golding concluded that consistent exercise over a long period of time, lowered the serum cholesterol to a greater degree than sporadic or erratic exercise for a shorter period of time.

The possibility of physical activity having a prophylactic effect on serum cholesterol increase was further supported by Rochelle (1961). Plasma cholesterol levels were followed in six experimental and six control subjects during a five-week exhaustive exercise program (timed two-mile run, five days per week and an eight-week detraining period). The plasma cholesterol concentrations were significantly reduced during the course of intensive training. A temporary rise in plasma cholesterol during the exercise phase occurred, which was believed to be due to fat mobilization and ultimate utilization during physical exercise. Four weeks after the detraining period, the plasma cholesterol levels returned to the pre-training levels.

A study was conducted by Naughton and Balke (1964) on 92 male physicians and medical students which were divided into the following groups: (i) sedentary individuals, (ii) active (those who had a history of competitive athletic participation), and (iii) sedentary, trained individuals (sedentary subjects who participated in an exercise program and determined trained when they could run 3 miles in 24 to 28 minutes). The exercise test applied to groups two and three was the maximum work capacity test. The subjects began walking on a one per cent treadmill grade at 3.4 mph and the grade was elevated one per cent each minute. The exercise period was terminated when a pulse frequency of 180 beats per minute was attained or with

the onset of severe dyspnea, fatigue or claudication. Group one had a mean serum cholesterol concentration of 232 mg per 100 ml, whereas group two had 208 mg per 100 ml. Before the physical training period, group three had a mean serum cholesterol concentration of 260 mg per 100 ml, while the post-training value was 210 mg per 100 ml. Therefore, the hypothesis that exercise has a hypocholesteremic effect seems valid.

To determine the influence of several types of physical activities upon the serum cholesterol, Campbell (1965) randomly selected 133 male freshmen college students to participate in 10-week programs of cross-country running, golf, tennis, tumbling-gymnastics, wrestling, and weight training. Only cross-country running and tennis produced a significant serum cholesterol reduction. This study illustrated the necessity of regular, vigorous physical activity in order to induce a change in serum cholesterol concentration.

The following year, Campbell (1966) reported the influence of diet and physical activity on serum cholesterol of 86 young men who were divided into six categories; lean, muscular, and obese individuals who were either physically active or inactive. The active subjects ran from 5 mph at 0 degree elevation to 7.5 mph at 10 degrees on a treadmill three times a week for ten weeks. There was a significant difference in serum cholesterol level between the active and inactive groups. The

greatest serum cholesterol reduction occurred in the obese, active subjects. The reduction of serum cholesterol was independent of dietary influences and weight changes.

A group of 229 Air Force officers who routinely engaged in a phasic or dynamic exercise program for at least one year was compared, for serum cholesterol differences, against 126 officers who were classified as sedentary (Hoffman et al., 1967). The exercise group had significantly lower levels of total lipid, cholesterol, beta-lipoprotein, and triglycerides.

Similarly, Carlson and Fröberg (1967) not only found a significant decrease in plasma concentration of cholesterol, but also in phospholipids and triglycerides in 12 men who walked 500 km in 10 days. The interpretation of the effect of physical activity on the change in cholesterol concentration is difficult since the subjects went without food for the entire period. They subsisted on mineral water, fruit and vegetable juices, estimated to be about 200 kcal per person per day.

Negative correlations      Twelve male varsity swimmers were studied over a period of 14 months with regard to the effect of a typical training and competitive collegiate swimming program on plasma cholesterol (Johnson and Wong, 1961). The workout consisted of swimming one to two miles daily in a series of 440-yard or 220-yard sprints. The rest interval was from 5 to 10 minutes between sprints. The daily caloric intake was moderately low, averaging 2559 kcal. It was

concluded that exercise, in the form of varsity swimming, did not significantly lower blood cholesterol. Closer examination of the data revealed that the initial serum cholesterol levels of the college swimmers had a mean pretraining value of 169 mg per 100 ml, which was relatively low for that age group (19.3 years). A study by Schilling et al. (1964) indicated that the average serum cholesterol concentration for that age was 193 mg per 100 ml. Therefore, the pretraining cholesterol values may represent the lower limits of the physiological range, and to depress that level even further would require greater physical effort than would be the case for a hypercholesteremic group. As with Rochelle (1961) and Naughton and Balke (1964), the authors of this study reported that the blood cholesterol increased with the onset of muscular exercise. They suggested that the body may have a high priority for lipids, including cholesterol, for fuel to supply the increased metabolic needs resulting from acute physical exertion.

Brumbach (1961) studied changes in serum cholesterol levels of 40 freshman students from the University of Oregon who failed the University's physical fitness test. Twenty of these subjects remained sedentary (controls). The remaining persons volunteered to take part in an exercise program which met for 35 minutes three times a week for 10 weeks. Exercise included the U.S. Army Conditioning Exercise Drill No. 1, running (starting with 440-yard jogs and gradually increasing



the distance to 740 yards) and weight lifting. No significant difference in serum cholesterol levels was detected between the control group and exercised group. However, the investigator questioned the "vigorousness" of the employed physical exercise program.

Holloszy and his associates (1964) determined the effects of a six month program of endurance exercise on the serum lipids of middle-aged men. One group of 15 men (average age = 41.7 years) who previously led sedentary lives for three or more years participated in a progressively strenuous program of endurance calisthenics and distance running (2 to 4 miles), averaging 3.35 times per week for six months. Twelve men in another group (mean age = 42.5 years) who also had been sedentary for a number of years, took part in a program of distance running geared to their individual capacities and which increased progressively in intensity. It was estimated that the men expended approximately 1,000 kcal per week from participating in the exercise program. Caloric intake was about 2,596 kcal per day per individual. No significant change between the initial serum cholesterol level and the post-training level was found in either group. In contrast, there was a significant decrease in the mean fasting triglyceride levels in both groups. Apparently this reduction in serum triglycerides occurred within two to three hours after exercise and lasted for about two days. There was some indication that

this reduction may be cumulative, an observation first to be recognized and reported. Similar results were attained by Carlson and Mossfeldt (1964) who investigated plasma lipid levels of normal persons who participated in the 1962 and 1963 "Vasaloppet" (an annual ski-racing event). Training consisted of indoor gymnastics five months before the experiment. The most pronounced decreases of the plasma lipids was in the triglyceride fraction, and this decrease were directly and significantly correlated to the fasting triglyceride concentration. Approximately 75 per cent of the decrease in triglyceride concentration was due to a reduction in the amount of triglycerides in the very low density lipoproteins. No significant changes were observed in the cholesterol content of any of the lipoproteins.

Fasting total lipids and serum cholesterol levels were studied in 15 medical students, three research workers and two laboratory technicians who participated in 14 days of consecutive training of short bouts of strenuous exercise (Fitzgerald et al., 1965). The exercise regimen consisted of nine sets of different calisthenics, each of 60-second duration, with 30-second rest intervals. The results showed that the course of training did not alter the fasting total lipids and serum cholesterol. According to Åstrand and Rodahl (1970) the average caloric output due to calisthenics is approximately 4.5 kcal per minute. Therefore, about 39.5 kcal were expended per training session, a value that can hardly be classified as

"strenuous", let alone beneficial. That amount of "work" can be accomplished by milking a cow by hand for eight minutes (Åstrand and Rodahl, 1970). The point is that conclusions are questionable when the intensity and duration of exercise are in question.

Wilcox et al. (1964) studied serum lipid concentrations associated with protein, milk intake and exercise on 24 university athletes representing the competitive sports of football, basketball, track and field, and wrestling. All subjects were in training for, or were participating in, their respective sports during the entire investigation. Seven basic menus were created, all of which contained 35 per cent of the calories as fat. The menus were adjusted for protein (10.0, 13.4, and 16.8 per cent of the total calories) and milk content (no milk, one quart and 2 quarts per day per individual) to meet the specifications of each diet. The caloric intake was adjusted to maintain the weight of each subject, and required a range of 3,100 to 4,700 calories. No significant effects of the various diets or exercise were evident as measured by the serum cholesterol, total lipid, or phospholipids. Although no hypocholesteremic effect was evident through the exercise program, this need not be interpreted as a failure of exercise to have a therapeutic effect in maintaining or lowering serum cholesterol level. This is especially so when one considers the hypercholesteremic diet

given to the athletes (high caloric intake, two eggs per day, high amounts of saturated fats and large quantities of milk).

It has long been known that milk will induce a significant rise in blood cholesterol level (Beveridge et al., 1956; Anderson, 1959; Keys et al., 1957; Okay, 1959). Closer examination of the data reveals that in no instance did the serum cholesterol level rise above the initial serum cholesterol concentration even when the special diets were given during the training period. Therefore, one cannot neglect the possibility that the training prevented an increase in serum cholesterol concentration, due to a possible hypercholesteremic state induced by the diet. By the same token, this may be interpreted to mean that exercise had a greater effect on serum cholesterol concentration than did the diet supplemented with milk-protein.

#### Animal experimental research

The beneficial effects of physical activity in lowering serum cholesterol concentration are more evident in laboratory animal studies. This can be attributed, primarily, to a more homogenous sample subjected to a more controlled and symmetrical treatment than the subjects in epidemiological and human experimental studies.

Positive correlations      Brown et al. (1956) were the first to report the possible salutary effect of exercise in lowering serum cholesterol. Rabbits were divided into six groups: (i) basal diet, no exercise; (ii) basal diet,

exercised; (iii) 0.1 per cent cholesterol, no exercise; (iv) 0.1 per cent cholesterol, exercised; (v) 0.5 per cent cholesterol, no exercise; and (vi) 0.5 per cent cholesterol, exercised. A 12-week exercise program consisted of 20 minutes of daily compulsory running in a large, cylindrical, motorized barrel which revolved 12 to 15 times per minute. The results in the six groups were as follows: 36, 42, 350, 192, 1134, and 581 mg cholesterol per 100 ml respectively. Serum total cholesterol was increased as the levels of dietary cholesterol were increased. Although no effect was found in the exercised group on basal diet, a hypocholesteremic effect was found in the exercised groups receiving 0.1 and 0.5 per cent dietary cholesterol.

A similar experiment was conducted by Wong and his associates (1957) on cockerels. The birds were separated into three categories: (i) plain mash diet, no exercise (control group), (ii) 2 per cent cholesterol + 5 per cent cottonseed oil, no exercise; and (iii) similar dietary regimen as group ii but exercised. Two daily sessions on a motorized treadmill for seven weeks was the method employed for physical activity. A significant difference was observed in the serum total cholesterol level of group one when compared to group two or three; and a marked difference in concentration had resulted in group two when compared to group three. In addition, there was a subsequent reduction in the formation of atheromatous plaques of the abdominal aorta of the exercised birds. This

is in agreement with their earlier finding (Wong et al., 1956).

In 1957, Orma reported an extensive investigation on serum lipid levels of 145 cockerels which were classified into four categories: active and inactive on ordinary diet; and active and inactive on 1.5 per cent cholesterol-supplemented feed. The birds were rendered "inactive" by restricting the pen area to allow minimal movement. The "active" birds were allowed a large pen to provide ample motion. It was concluded that no difference appeared in either the serum lipid values or the degree of atherogenesis between the active and inactive control groups fed ordinary diet. In the cholesterol-fed groups the lipid values of the inactive group were significantly higher than those of the active cholesterol-fed group. Moreover, the incidence of atherosclerosis was higher and its severity more marked in the former group. Also, by measuring the relative colloid volume in the thyroid, a much higher volume was found in the active, cholesterol-fed birds as compared to the inactive. This was interpreted as increased thyroid activity, which was the first inference of its kind relating the possible increase thyroid activity associated with exercise.

In another study (Myasnikov, 1958) there was a marked reduction of blood cholesterol in rabbits that were trained to run on an electric-driven treadmill until signs of marked fatigue appeared in comparison to those receiving only the cholesterol-supplemented feed.

Rabbits consuming a basal diet had a serum cholesterol concentration of 59 mg per 100 ml, whereas those on a 0.3 per cent cholesterol-supplemented diet had a mean concentration of 903 mg per 100 ml (Brainard, 1959). A cholesterol fed group who exercised in a motorized-drum (alternate periods of rest and exercise every 15 minutes) for eight hours daily for two months experienced a pronounced hypocholesteremic effect, 501 mg per 100 ml.

Kobernick and Niwayama (1960) reported that rabbits given a high-cholesterol diet (750, 250, and 150 mg per day) had a lower serum cholesterol when exercised only if they were "adequately" exercised. No difference was found in the group of rabbits that had insufficient amount of physical activity. Moreover, in the adequately exercised group (induced to run continuously in a drum by periodic electrical stimulation) the amount of atherosclerosis in the aorta was distinctly smaller than the sedentary controls.

An extensive study by Lewis and co-workers (1961) on the lipid concentration in serum and tissues was studied in four groups of 100 rats each fed high fat diets containing 22 or 54 per cent saturated coconut oil or unsaturated soya oil. Another 185 rats were fed Hartroft diets, which is a high saturated fat diet (69 per cent of total calories from fat) containing 0.3, 1.0, or 3.0 cholate. The 75 chow-fed rats served as controls. Approximately half of each group were

exercised on a treadmill eight hours daily in which the drum alternately revolved for 2 minutes and stopped for one minute. The exercise period lasted for 9 to 14 weeks. Exercise was effective in reducing serum total lipid and cholesterol concentrations only in those animals in which the levels were elevated by the high fat intake. When hepatic lipid concentrations were normal, as in rats fed chow diets, exercise had no effect. In rats fed the Hartroft diets, serum but not hepatic lipid accumulation was decreased by exercise.

Montoye et al. (1962) investigated the effects of exercise on serum cholesterol and correlated those effects with estimates of body fat through experiments with 45 albino weanling rats (15 litter mate trios). Group A was fed a stock diet and exercised for an hour in the morning and an hour in the afternoon by forced swimming five times a week for 12 weeks. Group B was subjected to the same exercise but a portion of the stock diet was replaced with powdered whole milk so that 30 per cent of the caloric intake was derived from the whole milk supplement. Group C was fed the stock diet but activity was restricted. The total serum cholesterol concentrations at the end of the experimental period were 74.83, 84.90, and 93.08 mg per 100 ml. This clearly indicated that physical activity had a definite hypocholesteremic effect upon the basal diet group as well as the high fat diet group subjected to swimming two hours daily for 12 weeks. The final body weight was less in



the exercised group. The additional weight in the nonexercising group was partially due to fat as indicated by the significantly higher specific gravities of the carcasses in the exercised group.

The effect of 15 weeks of regular, vigorous exercise (swimming) on serum and hepatic cholesterol of rats was studied by Jones et al., 1964. The experimental design included four groups: (i) young unexercised rats 13 weeks old, (ii) sedentary adults on stock diet, (iii) exercised adults on stock diet, and (iv) sedentary adults on a caloric-restricted diet to maintain their weights as closely as possible to the weights of those of the exercising animals in group three. The caloric-restricted animals were retarded in growth as evidenced by lower ash weight but their body composition on a percentage basis was almost identical to that of group two. It was concluded that exercise was effective in preventing most of the increase in body fatness and serum cholesterol associated with increasing age. Neither total nor free cholesterol concentration in the liver was affected by physical activity, but the concentration of total hepatic lipids was reduced.

The effects of training programs of various intensities, detraining and terminating training on liver cholesterol levels of rats maintained on high-fat diets were studied by Gollnick and Simmons (1967). The effect of exercise on the excretion of cholesterol via the feces was also studied. In the first

experiment 52 rats with an average initial body weight of about 320 grams were divided into four groups equalized with respect to body weight. Group A, B, and C swam 15, 30 and 60 minutes per day, respectively. Group D served as unexercised controls. Hepatic cholesterol (mg/g wet weight) was 3.06, 3.41, 3.19, and 4.95 respectively, which indicated that the nonexercised animals had the highest concentration. Increasing the daily exercise period beyond 15 minutes did not produce any further statistically significant reduction. Free plasma cholesterol was not altered by training, while total plasma cholesterol was significantly lower only in the group of rats exercised 60 minutes per day (81.91, 78.15, 68.41, and 83.38 mg per 100 ml respectively). Animals which exercised excreted significantly more sterol in their feces than those which did not exercise. The lack of any difference in sterol excretion between the three exercise groups agrees with the pattern observed for liver cholesterol levels. In the second experiment, rats were trained to run in motor-driven work wheels. The speed and duration of the daily exercise bout were progressively increased until the animals were capable of running for 60 minutes at 1.0 mph. At the end of eight weeks, one group of rats was taken off the training program while a second group was progressively detrained for four weeks by reducing the exercise two minutes each day. A third group of rats continued the daily 60 minute run throughout the entire experimental period. Unexercised animals served as controls for the fourth

group. Plasma cholesterol was not significantly altered by any of the training programs in which running was used as the exercise. The concentration of cholesterol in the livers of the trained group was significantly lower than that of the untrained group. This change also appeared to be temporary since the difference between the control group and group that terminated training at the end of eight weeks was not significant. The detrained group also appeared to have increased. It was concluded that the increased excretion of cholesterol via the feces of the exercised rats is one of the means by which the lower hepatic cholesterol level was maintained.

Analogous results on increased cholesterol excretion via the feces in mice were reported by Hebbelinck and Casier (1966). The daily bouts of training which were considered exhaustive consisted of running on a miniature treadmill. Intra-peritoneal injection of 4-<sup>14</sup>C was administered to the animals in order to examine the effects of exercise on the quantitative distribution of sterol and saponifiable fractions in the liver and excreta. The results were interpreted to indicate that muscular exercise can stimulate the conversion of cholesterol to bile acids and enhance its rate of excretion into the feces. It was inferred that physical activity may be assumed to increase the rate of cholesterol catabolism. The lower cholesterol content in the liver of exercising animals confirmed other reports (Lewis et al., 1961, Gollnick and Simmons, 1967; Gollnick, 1963; Simko and Babala, 1964). The data

seemed to indicate that it was doubtful whether short-duration or single activity might be sufficient to increase the cholesterol catabolism because the differences in excretion of acidic materials and neutral sterol started only after the sixth day of exercise. Only when physical exercise was continued over a longer period was there evidence that cholesterol catabolism and excretion was enhanced.

The effects of strenuous exercise and training were studied on plasma triglyceride, nonesterified fatty acid (NEFA), cholesterol, phospholipids, and lipoprotein in rats (Papadopoulos and collaborators, 1969). Exercise consisted of four hours swimming which lasted for four weeks. Another group of animals was subjected to the same exercise treatment but had a pre-training period to alleviate the possibility of psychic stress due to forced swimming. It was Kratzing (1965) who indicated that rats that stood in water for 2-1/2 hours had hepatic lipid and cholesterol values comparable with those which swam the same length of time. He concluded that factors other than muscular exercise were involved. Similarly audio-visual stress (Anderson et al., 1965), emotional stress (Wolf et al., 1962; Chapman and co-workers, 1966), psychic stress (Lang, 1967), and electrical shocks (Uhley and Friedman, 1959) have been known to alter serum lipid and cholesterol concentrations. However, such effects were not demonstrable by loud generator noises (Anthony and Babcock, 1958). Horst et al.,

(1960) were not able to duplicate the results of Uhley and Friedman (1959) by electric stimulation. The results of Papadopoulos's et al. (1969) experiment indicated that a sufficiently strenuous exercise and training program may lead to a lowering of serum cholesterol, triglyceride, and certain lipoprotein levels in rats. It appeared that there was a relation between the degree of training and the extent to which the plasma cholesterol was lowered, as there was a continuous decrease throughout the exercise period. The apparent rise in plasma cholesterol observed at the end of the first week in the untrained group could possibly be related to psychic stress. However, at the end of the experimental period, the hypocholesteremic effect in both the trained and untrained group forced to swim had comparable values. On the other hand, the NEFA was significantly higher and triglycerides significantly lower in the untrained group when compared to the trained group at the end of four weeks.

Ahrens and Broxton (1970) compared three levels of forced swimming (lead weights 2, 4, and 6 per cent of the body weight attached to the tail) to the sedentary controls (immersed in water up to their necks). The young male adult rats were fed a high-fat diet containing 38 per cent of the calories from protein, 50 per cent from fat, and 12 per cent from one of two carbohydrate sources, cornstarch or a mixture diet representative of the typical U.S. "market basket" diets. It was found

found that exercise (daily bouts of 10 minutes of exercise for 60 days) increased serum cholesterol concentration in the starch fed group but decreased the concentration in the mixture diet group that was forced to swim with weights equivalent to six per cent of their body weight (BW). This group was classified as exhaustively exercised. The control group (immersed) that was fed the mixture diet had a significantly increased blood cholesterol concentration. Equally confusing was the fact that the moderate level of exercise (2 per cent loading) led to the lowest body weight, the lowest rate of increase in tissue cholesterol, and the lowest accumulation of lipophilic material in the aorta. These results can be attributed to the low water temperature (27°C) to which the rats were subjected during the swimming exercise program. This may have induced a hypothermal stress or some other unnatural physiological phenomenon. Baker and Horvath (1964) studied the influence of water temperature on oxygen uptake of swimming rats and found that the animals swimming in cold water were exhausted before their oxygen uptakes stabilized. Wilber and Hunn (1960) reported that there was a positive relationship between decreasing water temperatures and swimming time to exhaustion. Therefore, the performance of the rats, especially those with the heavier load (6 per cent), may have been due to hypothermic stress, oxygen deficiency, decreased swimming time as compared to the lighter weight-loaded rats, and/or altered

metabolism as an adaptation to the metabolic cost of thermogenic activity.

The effects of training on plasma and tissue lipid levels of aging rats was reported in 1969 by Carlson and Fröberg. One group of 10 male rats (12-13 months old) was trained on a treadmill by running at a speed of 30 cm/sec, 3 hours a day, 5 days a week for 3 weeks. Electrical shocks were employed to induce the rats to run. Another group of 10 rats served as controls. The type of diet was not mentioned, but I assumed it to be basal. The high concentration of serum cholesterol of the controls (249 mg/100 ml) as compared to the trained rats (186 mg/100 ml) indicated that the hypocholesteremic effect (reduction of 75 per cent) was highly significant.

Recently Malinow and collaborators reported that when cholesterol-26-<sup>14</sup>C is injected intravenously into an animal the recovered <sup>14</sup>CO<sub>2</sub> in the expired air, it can be used as a quantitative index of cholesterol side-chain degradation. It was found that treadmill running accelerated cholesterol degradation in rats (Malinow et al., 1968a) and in man (Malinow and Perley, 1969). Muscular contraction via electrical stimulation of the hindlimbs produced similar results in rats and squirrel monkeys (Malinow et al., 1968b). In 1969, it was reported that the oxidation was highly dependent on the adrenal glands, both during rest and exercise, and it was postulated that the hypocholesteremic response to

exercise may be mediated through the adrenal glands, which can increase the rate of cholesterol side-chain cleavage (Malinow and co-workers, 1969). It was estimated that roughly 80 per cent of the cholesterol oxidation depends on the presence of the adrenal glands in the initial periods of exercise and 20 per cent in the later period. Their latest study on hepatectomized and adrenalectomized rats (Malinow et al., 1970) seemed to support their thesis that the adrenals as well as the liver were mainly responsible for splitting the side-chain of cholesterol during rest and during muscular stimulation.

How important are the adrenal glands in promoting increased cholesteroleresis due to the increased physical activity? There are some indirect indications that the adrenals may play a role in the hypocholesteremic effect of exercise. There are ample reports on increased adrenal size in rats forced to exercise (Donaldson, 1932, 1933, 1935; McClintock et al., 1939; Kimeldorf and Baum, 1954; Hearn and Wainio, 1956; Wong et al., 1957; McArdle and Montoye, 1967; Tipton et al., 1968; and Ring et al., 1970). There is some evidence that prolonged physical exercise may stimulate the adrenocortical activity in rats (Frenkel and Csalay, 1962), dogs (Suzuki, 1966), and man (Bellet et al., 1969), whereas in other investigations no such effect was found (Renold et al., 1951; Hill et al., 1956; Connel et al., 1958; and Papacostas et al., 1960).



Negative correlations

In 1957, Kobernick and collaborators reported the effects of physical activity on cholesterol-fed rabbits (28 grams/day). The animals were electrically stimulated to run on a treadmill (50 rpm for 5 minutes, twice daily for a period of 4 weeks). There was no difference in the serum cholesterol and phospholipid concentrations between the sedentary and exercised group.

In contrast to their earlier study (Wong et al., 1956 and 1957) Wong and his associates (Wong et al., 1960) found no significant effect of physical activity on blood cholesterol, aortic and coronary atherosclerosis of 45 week-old, egg-laying hens fed an atherogenic diet of 2 per cent cholesterol and 5 per cent cottonseed oil. After 12 weeks the nonexercised hens had the highest incidence of aortic atherosclerosis and marked elevation of blood cholesterol when compared to controls on plain mash diet. The exercised birds which were on an atherogenic diet did not show a decrease in aortic atherosclerosis or show any change in blood cholesterol level.

The serum hypocholesteremic effect of four groups of rats that were fed two different diets was examined by Gollnick (1963). The effect of training (one-half hour daily swimming for 22 weeks) was also examined on serum cholesterol levels. The groups were divided as follows: (i) basal diet, untrained; (ii) basal diet, trained; (iii) 1.0 per cent cholesterol-0.5 per cholate diet, untrained; and (iv) 1.0 per cent cholesterol-

0.5 per cent cholate diet, trained. The data revealed that training significantly lowered total fat and serum cholesterol in group two, but produced a rise in both lipid components in the cholesterol-cholic acid fed group.

The effect of fat intake and exercise on serum cholesterol was reported (Hanson et al., 1967) on four groups of rats receiving a high-fat diet and four groups continuing to receive the low-fat diet. Each of the two groups were subdivided into two more groups in which one group was fed ad libitum and the other fed 65 per cent of the ad libitum group. These four groups, which were trained, had sedentary controls. The exercised groups were forced to swim for two 30-minute periods each day for six weeks. Their results showed that serum cholesterol was higher in caloric-restricted than in ad libitum fed animals and higher in exercised than in sedentary animals. Serum cholesterol was highest in caloric-restricted animals forced to exercise. There appeared to be a trend toward higher serum cholesterol in rats fed the high-fat diet as opposed to the low-fat diet. Exercise had no effect on either group. It was suggested that the utilization of fat for energy results in accelerated cholesterol biosynthesis. The animals restricted in calories and forced to exercise would have used the greatest amount of body fat for energy, thereby increasing cholesterol biosynthesis which then, affects the serum cholesterol level.

## MATERIALS AND METHODS

This study was designed as a 4 x 5 analysis of variance factorial experiment in which each of the 20 treatment combinations contained 12 male rats (Sprague-Dawley-Rolfsmeyer) weighing  $220 \pm 20$  grams at the initiation of the investigation. The main and simple effects of four levels of physical activity and five levels of thyroid status on the final body weight, feed intake, and serum and hepatic total cholesterol concentrations were determined.

Subcutaneous administration of L-T<sub>4</sub> (Na-salt, L-thyroxine) in physiological amounts<sup>1</sup> (established from the study of Kumaresan and Turner, 1967) to the 70-day old thyroidectomized<sup>2</sup> rats were performed daily for 10 weeks. The following thyroid states were produced: (i) athyroidism, no L-T<sub>4</sub> replacement therapy; (ii) hyperthyroidism, 3.5 ug L-T<sub>4</sub>/100 g BW; (iii) euthyroidism, 1.0 ug L-T<sub>4</sub>/100 g BW; (iv) hypothyroidism, 0.5 ug L-T<sub>4</sub>/100 g BW. The fifth group, rats with intact thyroids, served as (v) euthyroid controls.

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<sup>1</sup>See Appendix for the procedure for making L-T<sub>4</sub> solutions: stock, 3.5, 1.0, and 0.5 ug/0.1 ml.

<sup>2</sup>The thyroidectomies were performed on 60-65 day old rats in this laboratory. The surgically thyroidectomized rats were allowed to recuperate until day 70 when experimental treatment began.

Each of the above five levels of thyroid activity were further subdivided into four levels of physical activity:

(i) nonexercised controls, (ii) standing in three inches of water for 15 minutes to check the possibility of induced stress by water as was reported by Kratzing (1965), (iii) moderately exercised, and (iv) exhaustively exercised. The criterion for rats swimming until exhausted (Hardin, 1965) was allowing each rat to swim until it could no longer remain swimming above water (10 seconds of submergence). To decrease the swimming time and to stimulate more vigorous swimming, lead weights (4 per cent of BW) were attached to the tail; and to reduce the buoyancy effect of trapped air under the fur, a wetting agent was added to the water. Moderate exercise was arbitrarily established as one-half the mean weekly swimming time of the respective exhaustively exercised groups.<sup>1</sup> Since McArdle and Montoye (1966) have demonstrated that pre-training periods significantly improved the reproducibility of the swimming time to exhaustion, a one-week pre-training period was enforced by increasing the weight load 0, 2, and 4 per cent of the body weight (BW) every second day. The animals were individually swum in 20 gallon plastic tanks (53 cm deep x 43 cm wide) six days a week for 10 weeks. Water temperature was maintained at  $36 \pm 1^{\circ}\text{C}$ .

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<sup>1</sup>For mean swimming time of the exhaustively exercised groups, see Appendix, Table 9 and Figure 8.

Drinking water and food (Wayne Lab-Blox basal diet) were provided ad libitum to all 240 rats in which measurements of feed intake were accomplished daily. Each morning the remaining pellets on the cage floor of each cage were recovered. The pieces of pellets (and crumbs) that fell through the wire-meshed cage floor on to the newspaper-lined recovery trays were carefully gathered and weighed with the rest of the remaining pellets of that cage. Daily feed intake was calculated by taking the difference of the remaining feed that was measured that morning from the previous day's weighed-input of feed of the respective cages. The total daily feed consumption of each cage was divided by the measured body weight of the rat that day and expressed the value as grams of feed intake/100 g BW/day.

The basal diet<sup>1</sup> was composed of 24.52 per cent protein, 4.15 per cent fat, 3.20 per cent fiber, and 8.45 per cent ash by weight. Total cholesterol content of the feed was approximately 0.28 per cent (Story and Griffith, 1971).

The rats were weighed every other day. Room temperature was maintained at  $24 \pm 2^{\circ}\text{C}$  and artificially illuminated (14 hours dark and 10 hours light per day).

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<sup>1</sup>J. I. Long, Wayne Feeds Research Division, Allied Mills, Inc., Libertyville, Ill. Data from chemical analysis. Private communication. 1968.

At the termination of the 10-week exercise period the rats were bled and then sacrificed. At this time a post-mortem check for remaining thyroid tissue was performed in the surgically thyroidectomized animals. Blood samples were taken from the tails of ether-anesthetized rats. The prepared sera were frozen ( $-20^{\circ}\text{C}$ ) until analyzed for serum total cholesterol by the AutoAnalyzer, as described in the AutoAnalyzer Method File, Cholesterol N-24a (Technicon Instruments Corporation, 1965). The livers were quickly removed, blotted, weighed, and frozen in liquid nitrogen. Each liver was individually sealed in plastic Kapak<sup>1</sup> pouches and kept frozen at  $-20^{\circ}\text{C}$  until total hepatic cholesterol determinations were made. Chemical analyses consisted of homogenizing 250 mg samples of liver in 9.75 ml isopropanol as described by Naito and Griffith (1971a) and analyzing the hepatic cholesterol content of the extracts by the method of Naito and Griffith (1971b)<sup>2</sup>. All serum and hepatic samples were extracted in duplicate and each were analyzed twice, thus providing quadruplicate samples for total cholesterol for each rat.

The data were tabulated and analyzed by the Statistical Laboratory at Iowa State University, Ames, Iowa. Analysis of variance was used to determine main effects, simple effects,

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<sup>1</sup>Kapak Industries, Inc., 9809 Logan Avenue, Bloomington, Minn.

<sup>2</sup>See Appendix for procedure.

and interaction of the two parameters (physical activity and thyroid status) of the 4 x 5 factorial experiment. Analysis of covariance was utilized to determine the influence of feed consumption (by adjusting for the variability of the intake of feed) on the cholesterol concentrations with the different treatment combinations. The Student's "t" Test was used to determine the significance of the simple and main effects (Snedecor and Cochran, 1967).

## RESULTS AND DISCUSSION

The results of this investigation are tabulated in Tables 2 to 11 and are graphically represented in Figures 4 to 8. For convenience the groups of rats were coded as groups I to XX (Table 1).

The Effects of Thyroid Status  
and Physical Activity

Final body weight

The results of the analysis of variance indicated that both thyroid status (A) and physical activity (B) had a highly significant influence ( $P < .0005$ ) on the final body weight of the animals at the termination of the experimental period (Table 10). Interaction of the two parameters (AB) was found to be nonsignificant.

The main effect<sup>1</sup> between the athyroid (A-Tdx) group and control group with intact thyroids (C) was found to be highly significant ( $P < .0005$ ). The former group had a mean final body weight  $(294.9 \pm 16.3 \text{ g})^2$  that was 27 per cent lower than

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<sup>1</sup>Main effect is the averaged simple effects. Simple effects are the differences of each of the treatment means of a particular level to the respective treatment means of another level. The Dunnett's Test was used to compare a treatment combination at one level to the control group of that level of treatment or factor.

<sup>2</sup>Mean  $\pm$  standard error of the mean (S.E.M.).



the controls' weight ( $400.3 \pm 9.1$  g). Similarly, the "physiologically"-induced-hyperthyroid (Hyper-Tdx) rats had a mean body weight (BW) of  $376.8 \pm 1.2$  g which was approximately 12 per cent lower than the controls' ( $P < .01$ ). The main effects of the hypothyroid (Hypo-Tdx) and thyroidectomized-treated-euthyroid (Eu-Tdx) groups were found to be non-significant when compared to the control. This is in agreement with other investigators (Grossie and Turner, 1965, 1961; Ellefson and Mason, 1962; Dayton et al., 1960). Gross observations on post-mortem carcasses in this investigation indicated that all A-Tdx rats had little epididymal fat and had shorter nose to tail lengths (Table 6), whereas the other three groups (C, Eu-Tdx, and Hypo-Tdx) seemed to have had normal fat depots and nose-tail lengths. The Hyper-Tdx animals had normal nose-tail lengths but little epididymal fat. This indicates that the lighter weights may be representative of the fat-depleted tissues in both the A-Tdx and Hyper-Tdx animals.

It is interesting to note that while the daily feed intake of the four A-Tdx treatment combination means (groups V-VIII) were lower when compared to the controls ( $P < .0005$ ), the feed consumption of the Hyper-Tdx groups was the highest of all the levels of thyroid status ( $P < .0005$ ). Apparently, even "physiological" dosages of  $L-T_4$  ( $3.5 \text{ ug}/100 \text{ g BW}$ ) was sufficient to increase the body metabolism to the level where fat accumulation was prevented in the Hyper-Tdx animals.

It has long been known that normal growth is dependent on the synergism of thyroxine and growth hormone (Beck et al., 1946) and that the feed intake in the rat is definitely influenced by the thyroid status of the animal (Grossie and Turner, 1961, 1965). Therefore, in the A-Tdx animals, the significantly lower body weight was mainly attributed to the stunted growth and low amounts of fat and muscle mass, while the lower weight in the Hyper-Tdx rats seemed to be primarily due to the loss of epididymal fat depots.

The influence of physical activity on body weight can be ascertained by comparing the means of standing (S), moderately (M), and exhaustively (E) exercised groups against the non-exercised controls (NC). There was no significant difference in the main effect of the S group ( $377.3 \pm 13.7$  g) when compared to NC ( $386.4 \pm 13.9$  g), but BW was significantly reduced in the M ( $P < .01$ ) and E exercised animals ( $P < .0005$ ). The mean final BW of the latter two groups were  $361.8 \pm 12.7$  and  $355.4 \pm 9.2$  grams, respectively. More specifically, the reduction in BW that occurred in treatment combinations IV, XV, and XVI was highly significant ( $P < .0005$ ) and groups III and XX approached statistical significance ( $.05 < P < .1$ ) when tested by Dunnett's procedure. No statistically significant change in BW was found in either the A-Tdx and Hyper-Tdx treatment combinations irrespective of the degree of physical activity.

It has been reported that the change in body weight due to chronic exercise is mainly represented in a quantitative lipid depletion. Jones et al. (1964) and Hanson et al. (1967) have shown a reduction of total carcass fat of 40 and 33 per cent respectively, of which greater than 80 per cent of the loss was from epididymal fat and the remainder from visceral fat of rats forced to exercise. It is conceivable that the fat-depleted tissues which characterize the A-Tdx and Hyper-Tdx rats in this study offered little opportunity for body weight reduction when the rats were forced to exercise. Conversely, significant reduction in final BW was found in treatment combinations IV, XV, XVI; and groups III and XX approached statistical significance. This tended to support the above explanation that physical activity can cause weight reduction providing that a sufficient amount of fat depots are available. The greater the excess available fat, the greater the reduction in body weight that can occur with increasing severity of exercise. The positive relationship between severity of physical activity and weight reduction can also be, in part, due to the decreased feed consumption which was found to be significant in E exercised rats ( $P < .02$ ) when compared to the nonexercised controls.

#### Daily feed intake

Contrary to the highly significant relationship of both the thyroid status and physical activity to final body weight,

daily feed intake was greatly influenced by the thyroid level ( $P < .0005$ ), and to a lesser extent by exercise ( $.025 < P < .05$ ). No significance was found on the interaction of the two factors, AB.

The averaged means of the A-Tdx groups ( $5.97 \pm .16$  g/100 g BW) and Hyper-Tdx groups ( $7.48 \pm .15$  g/100 g BW) were found to be highly significant when compared to the averaged means of C rats ( $P < .0005$ ). This is in agreement with Grossie and Turner (1965, 1961) who found that thyroidectomized rats of the same strain had a decreased food consumption while the thyroxine-treated rats (3, 6, 9, and 12  $\mu$ g L-T<sub>4</sub>/100 g BW/day) increased their consumption of daily feed. A similar direct correlation between feed intake and thyroid status was found in the Hypo-Tdx animals in this study. The daily feed intake in these rats was lower than C ( $P < .02$ ). The Eu-Tdx rats (groups XIII-XVI) had a daily feed consumption averaged means of  $6.29 \pm .15$  g/100 g BW which was a significant reduction when compared to the averaged mean of C ( $P < .001$ ). The difference in feed intake in the two groups of euthyroid animals, C and Eu-Tdx, could be ascribed to the decreasing circulating levels of L-T<sub>4</sub> in the latter group. While continued peripheral utilization of L-T<sub>4</sub> occurred in both groups, especially during exercise, euthyroid status was maintained in C, but the Eu-Tdx animals may have approached the limits of hypothyroidism due to the lack of continued thyroid secretion. The nonsignificant

difference of the averaged mean of Hypo-Tdx and Eu-Tdx tends to support this hypothesis.

When the averaged means of S, M, and E were compared to NE, the effect of physical exercise on feed intake becomes apparent. While the main effect of M was found to be nonsignificant when compared to C, the S and E were found to be significant ( $P < .05$ ). The significant effect of E is in corroboration with other data (Mayer et al., 1954; Johnson, 1956; Thomas and Miller, 1958; Stefanik et al., 1959; Miller and Payne, 1962; Stevenson et al., 1966; Mayer and Thomas, 1967; Stevenson, 1967). The decreased feed intake in exhaustively exercised rats was first recognized by Mayer and his associates in 1954, who demonstrated that, within the range of moderate activity, rats exercised on a treadmill increased their food consumption linearly while their weight was maintained. Rats exercised to exhaustion lost weight and their food intake decreased. Similar data has since substantiated Mayer's study. Thomas and Miller (1958) demonstrated that the maintenance of an essentially normal body weight in the exercised animals (despite an increased energy expenditure during exercise and the absence of a net increase in weekly food intake) was due to the decreased spontaneous activity on exercised days. Acknowledging the Law of Conservation of Energy (Kleiber, 1961), the decreased feed intake was met by a lower overall work output. Mayer and Thomas (1967) suggested that within a certain range of physical activity, the regulation of food intake is both precise and

sensitive. Any increase in physical activity is followed by a corresponding increase in food consumption. If activity is above or below this range, regulation of food intake becomes inaccurate. If the activity is too intense, the animals become exhausted, their food intake decreases, and their body weight drops. Similar results were found to be true in human studies (Johnson, 1956; Stefanik et al., 1959).

In contrast to the exhaustively exercised groups, the moderate exercised animals in this investigation showed no significant change in daily feed intake. This is not in agreement with Mayer et al. (1954).

The significant reduction in daily feed intake in S is in agreement with Kratzing (1965). Thomas and Miller (1958) and Stevenson (1967) believed that it is probable that unusual exercise such as forced swimming or running may have some of the effects of a nonspecific stress, and that some of the depression of food intake may be ascribed to this. Scrimshaw (1964) reported that stress may precipitate acute malnutrition and decreased food consumption; and Kratzing (1965) reported a lower feed intake in rats that stood in three inches of water for 2-1/2 hours daily as compared to the sedentary group. In the present investigation, rats that stood in three inches of water for 15 minutes, 6 days a week for 10 weeks had a lower feed intake ( $.02 < P < .05$ ) than the averaged means of the NC.

Therefore, the probability that forced-exercise alters feed consumption via overstimulation of either the parasympathetic or sympathicoadrenal system is not to be overlooked.

#### Serum total cholesterol

Analysis of variance on the two factors (A and B) denoted that the thyroid status, but not physical activity, had a significant effect on serum total cholesterol. AB interaction was found to be nonsignificant. When the 20 treatment combination means were adjusted to account for the varying feed intake by the use of analysis of covariance, factor A was still found to have a significant effect on serum and hepatic ( $P < .0005$ ) total cholesterol (Table 11). This indicated that feed intake was not an important factor in influencing the cholesterol levels in the two pools, serum and liver, when the rats were fed a basal diet. Also, this suggested that endogenous cholesterol was the primary constituent of the serum and hepatic total cholesterol concentrations. The unadjusted means were used when determining the effects of the thyroid status and physical exercise on serum and hepatic total cholesterol concentrations.

When compared to the averaged means of C ( $55.6 \pm 1.9$  gm/100 ml), the averaged means of A-Tdx ( $60.0 \pm 2.6$  mg/100 ml) and Hypo-Tdx ( $59.4 \pm 1.9$  mg/100 ml) groups were found to be significantly higher ( $P < .02$ ). This is in concordance with other investigators (Best and Duncan, 1956, 1959; Duncan and

Best, 1958a, b; Boyd, 1959, 1960a, b; Kritchevsky, 1960, 1967; Rosenman et al., 1952a, b; and Byers, 1958}. It has long been known that thyroid hormones stimulate the removal of cholesterol from the plasma, as was demonstrated in thyrotoxic rats and humans (Myant, 1964; Gould, 1959); and in other studies using labeled acetate (Tsung-chin and Shih-chen, 1965; Eskelson et al., 1970; Dayton et al., 1960), deuterium (Marx and co-workers, 1953), and tritiated water (Byers et al., 1952). Both of the main pathways on the disposal of serum cholesterol--its excretion as neutral sterol and its conversion to bile acids--are affected by thyroactive compounds (Siperstein and Chaikoff, 1952, 1955; Bergström and Sjövall, 1954; and Eriksson, 1957a, b). Conversely, suppressed thyroid function, by surgical excision of the gland, destruction by radioactive iodide, or the oral administration of antithyroid drugs elevates the serum cholesterol concentration by decreasing the plasma turnover rate, a reflection of the lowered rate of cholesterol catabolism and excretion.

The increase in serum total cholesterol concentration was found to be significant ( $P < .05$ ) in Eu-Tdx rats ( $58.1 \pm 2.4$  mg/100 ml) when compared to C. The difference between the two euthyroid groups, C and Eu-Tdx, was probably due to the decreasing concentration of the exogenous L-T<sub>4</sub> that was administered daily. With the half-life of L-T<sub>4</sub> being  $17.8 \pm 1.1$  hours in the euthyroid rat (Grossie et al., 1964) and with



the continued peripheral utilization of the hormone which may even be increased during exercise (Escobar del Rey and Morreal de Escobar, 1956; Irvine, 1967), the effective concentration of L-T<sub>4</sub> may have been lowered. This may have led to a lower turnover rate of cholesterol catabolism and excretion in the Eu-Tdx rats as compared to the controls which had intact thyroids to maintain the euthyroid status. This is reflected by the accumulation of cholesterol in the liver of the Eu-Tdx rats forced to exercise until exhausted as compared to the controls ( $.02 < P < .05$ ). Collectively, the Eu-Tdx groups (XIII-XVI) may have approached the lower limits of the "physiologically-euthyroid" state or upper limits of hypothyroidism. The comparable averaged means of this group ( $58.1 \pm 2.4$  mg/100 ml) to the Hypo-Tdx animals ( $59.4 \pm 1.9$  mg/100 ml) gives greater credence to the hypothesis.

A similar reduction in the concentration of the exogenous circulating L-T<sub>4</sub> of the Hyper-Tdx rats may have occurred (especially in the groups forced to exercise, groups XI and XII), but it was not great enough to change the hyperthyroid status of the animals. The serum total cholesterol concentration in these Hyper-Tdx rats may represent the minimum level at which serum total cholesterol exists under physiological conditions in rats fed a basal diet.

The analysis of variance and the comparison of the main effects of S, M, and E to the nonexercised control's ("t"-Test)

revealed no statistical difference on the serum cholesterol levels, but the simple effects were found to be significant in some groups when comparison of the means of the different treatment combinations were compared to their respective NC group (Dunnett's Test). In the four groups of rats, A-Tdx, Hyper-Tdx, Eu-Tdx, and Hypo-Tdx, physical activity did not produce any change in the serum total cholesterol concentration. In the C animals that were exercised to exhaustion (group IV), serum cholesterol reduction approached statistical significance ( $.05 < P < .1$ ) using the Dunnett's Test.

Considering the possibility that L-T<sub>4</sub> was continually being utilized by the animal and that this process was accelerated when increased peripheral utilization of L-T<sub>4</sub> occurred with increasing severity of exercise, it is reasonable to expect no reduction of the initially high serum cholesterol concentration with increasing severity of exercise in the A-Tdx groups (especially in groups VII and VIII). Since there was no exogenous or endogenous thyroxine to alter the cholesterol metabolism in the liver, the increased physical activity induced no change in the serum cholesterol levels in these groups of animals. The serum total cholesterol in these animals reflected the maximum plasma cholesterol concentration due to endogenous synthesis and the limited rate of catabolism and excretion of cholesterol by the thyroxine-dependent liver. This conjecture is also based on the accumulation of hepatic

total cholesterol in the A-Tdx groups as compared to C ( $P < .0005$ ).

The low initial levels of exogenous thyroxine that was administered daily ( $0.5 \mu\text{g}/100 \text{ g BW}$ ) to the Hypo-Tdx rats also produced no change in the level of serum cholesterol even in those rats subjected to severe exercise. It appears that even though small amounts of exogenous  $\text{L-T}_4$  were present, there may be a minimum saturation point of the binding sites on the mitochondria and microsomes which affects the effectiveness of cholesterol side chain degradation and levels below this concentration became ineffective. Where the exogenous concentration of  $\text{L-T}_4$  was initially high, as it was in the Hyper-Tdx animals, or when the endogenous concentration of  $\text{L-T}_4$  was maintained or even increased, as in the euthyroid control groups (groups III and IV), their blood cholesterol was lowered correspondingly--possibly by the increased fractional turnover rate of bile acid excretion.

The Hyper-Tdx treatment combinations (groups IX-XII) had averaged means of  $55.6 \pm 2.0 \text{ mg}/100 \text{ ml}$ . The Hyper-Tdx animals in the S, M and E groups had means that varied little from the NC group. It may be that, even with increased peripheral utilization of the circulating exogenous thyroxine in these groups of animals, the remaining amount was sufficient to combine with the active sites on the mitochondria and microsomes in a proportion that typified the rates of cholesterol synthesis and catabolism of hyperthyroid animals.

In contrast, the euthyroid controls demonstrated a tendency for serum total cholesterol to decrease with increasing severity of exercise. This suggests that the thyroid gland was necessary in order for exercise to have exerted its hypocholesteremic effect. While the reduction (group IV) only approached statistical significance ( $.05 < P < .1$ ), any further reduction becomes extremely difficult since the cholesterol concentration in this group represented the lower, "normal-physiological limits" of endogenous cholesterol concentration in the animals. Similar nonsignificant results of training on serum cholesterol reduction in rats on a basal diet was also reported by Lewis et al., 1961. Montoye and co-workers (1962) and Jones et al. (1964) reported a 20 per cent reduction in serum total cholesterol in male rats fed a basal diet and exhaustively exercised for 15 weeks. It is probable that exercising our rats for a five-week-longer period may have produced similar statistically significant hypocholesteremic effects due to exercise since the concentration of serum cholesterol have been reported to increase with age (Sperry and Webb, 1950; Carlson et al., 1968; Carlson and Fröberg, 1969). At the termination of the experimental period, our rats were 140 days old while those of Montoye et al. (1962) and Jones et al. (1964) were 310 days old and had a serum cholesterol concentration of about 95 mg/100 ml in the nonexercised group. Similar nonexercised controls in this present

investigation (group I) had serum total cholesterol concentration of  $57.8 \pm 2.1$  mg/100 ml. Therefore, even on a basal diet the rats in the studies of Jones et al. (1964) and Montoye et al. (1962) may have approached the "upper limit" of the normal serum cholesterol concentration for that age group or even entered a "physiological-hypercholesteremic state". This elevated range of cholesterol concentration could have then allowed greater degrees of cholesterol reduction due to forced exercise.

It is intriguing that groups III and IV had serum cholesterol values similar to their Hyper-Tdx counterparts, groups XI and XII. This may indicate that physical activity produces physiological hyperthyroidism in rats via increased TSR in the animals with intact thyroids. A number of recent investigations suggest this possibility. The effects of a single sustained physical exercise on the respiration of various tissues of rats were investigated by Romanowski and Strazynski (1968) by use of the Warburg technique. They found a highly significant increase in oxygen consumption of the skeletal muscle but not of the heart, liver, and spleen when rats were forced to run until signs of fatigue appeared (usually within 1-1/2 hours). Although increased respiration of the liver was not found to be significant, an increase did occur. Perhaps, a more significant  $Q_{O_2}$  of the liver could have been attained if the rats had been subjected to a prolonged exercise regimen

to allow structural and physiological adaptive changes. The increased  $\dot{Q}_{O_2}$  of the skeletal muscle may be supportive evidence that a state of physiological hyperthyroidism exists in severely exercised animals.

#### Hepatic total cholesterol

The main effects of A and B on hepatic total cholesterol concentration were found to be highly significant--significance being  $P < .0005$  and  $P < .001$  for thyroid status and physical activity, respectively. No interaction of A and B was found.

Although the importance of the liver in the synthesis, degradation, excretion, and regulation of blood cholesterol has been well established, the interpretation of cholesterol metabolism in the liver by mere concentration studies becomes particularly confusing because the literature is not in full agreement concerning the association between hepatic and serum cholesterol concentrations in different thyroid states.

Kritchevsky (1960) and Wells and Ershoff (1962) found no alteration in liver cholesterol levels in eu-, hyper-, and hypothyroid rats on basal diet or on a cholesterol-bile acid diet (Kritchevsky, 1967). When rats were fed a high-fat or high-fat-cholesterol diet, significant changes occurred in the serum and liver cholesterol concentrations; but, the results had little consistency on the relationship between the levels of the two cholesterol pools in the different thyroid states

(Kritchevsky et al., 1961). Similar data were presented by Marx et al. (1950) and by Dayton and co-workers (1954).

Contrary to these findings is the reported inverse relationship between serum and hepatic cholesterol levels with different thyroid treatments (Duncan and Best, 1958a, b; Fletcher and Myant, 1958; Best and Duncan, 1959; Ellefson and Mason, 1962).

In 1963, Patek et al. reported a positive correlation between the serum and liver cholesterol concentrations in hypophysectomized and thyroxine-treated rats.

Such inconsistent reports may be due to many variables: the type of diet, species differences, dose of the administered thyroxine, and the possible disturbance of normal cholesterol metabolism due to the addition of goitrogens, cholesterol, or bile acids to the diet. Moreover, the lack of change in the hepatic cholesterol concentration may not reflect the total picture of synthesis and degradation rates of cholesterol metabolism in the liver. Only by determining turnover rates of cholesterol as well as bile acid and neutral sterol output by the liver can the fate of the metabolic processes be depicted. This was demonstrated by Dayton et al. (1960). They found little fluctuation in hepatic total cholesterol concentrations in the different deranged thyroid states but found a parallel relationship on the turnover rates of acetate incorporation into cholesterol. The classical study of

Rosenman et al. (1952b) was one of the first to conclusively show that the hyperthyroid state was associated with a markedly increased rate of hepatic synthesis, destruction, and excretion of cholesterol. The decreased concentration of plasma cholesterol which occurred was a reflection of the differences in the balance of synthetic and catabolic rates of cholesterol by the liver, the latter metabolic process dominating the former. Conversely, the markedly depressed rate of hepatic synthesis of cholesterol found in the hypothyroid state was associated with hypercholesteremia because of the more marked decrease in the rates of destruction and biliary excretion of cholesterol also present in this derangement. Thus, the disparity between the altered processes of manufacture and elimination has been shown to underlie the inverse thyroid activity-blood cholesterol relationship. It appears that the major factor underlying the rise or fall of the blood cholesterol level in hypothyroid and in hyperthyroid states is the altered rate of turnover of cholesterol. A number of investigators have since substantiated their study (Thompson and Vars, 1953a, b, and 1954; Eriksson, 1957a, b; Zyl, 1957; Strand, 1963; Lin et al., 1963; Lepp and co-workers, 1964; and Miettinen, 1968).

In this present study the main effect of the A-Tdx rats, when compared to C, demonstrated a marked elevation in total cholesterol in the liver ( $P < .0005$ ), while no simple effects via the Dennett's Test were found with the different levels of



physical activity. This seems to indicate that normal cholesteroleresis is dependent on thyroxine. Without thyroxine the rate of hepatic cholesterol synthesis may have exceeded that of catabolism, thereby causing the accumulation of cholesterol in the liver as was found in all four A-Tdx groups. This hypothesis is substantiated by the work of Gruder et al. (1968) who found a 50 per cent lower HMG-CoA reductase activity in the liver of thyroidectomized rats as compared to the control. When compared with the control, cumulative fecal bile excretion was reduced about 20 per cent in the hypophysectomized and thyroidectomized rats, while the bile acid excretion approached normal rate in the hypophysectomized-thyroid-treated group (Beher et al., 1964). Therefore, while the biosynthetic rate of cholesterol decreased significantly in the athyroid rats, catabolism (which is dependent on thyroid hormones) decreased even further, causing an accumulation of cholesterol in the liver and leading to a concomitant rise in serum cholesterol level.

The animals in the athyroid state represented the minimum rate of cholesterol degradation and excretion by the thyroxine-dependent liver. Since there was no circulating thyroid hormone in all four treatment combinations (groups V-VIII), it is reasonable to expect that the hepatic concentration of cholesterol not be altered with increasing severity of exercise.

When compared to C, the accumulation of hepatic total cholesterol was found to be less, but significant, in the Hypo-Tdx animals (main effect) with simple effects being significant in group XX ( $P < .001$ ). Groups XVIII and XIX approached statistical significance ( $.05 < P < .1$ ). The rats in this group (Hypo-Tdx) were more effective in eliminating hepatic cholesterol than the A-Tdx animals but less effective than the other three groups (C, Hyper-Tdx, and Eu-Tdx). Moreover, the effectiveness of hepatic cholesterol catabolism and excretion decreased with increasing severity of exercise, due to the lack of thyroid gland and perhaps to the increased peripheral utilization of the exogenously administered L-T<sub>4</sub> (0.5 µg/100 g BW).

Grossie et al. (1964) reported that the biological half-life of L-T<sub>4</sub> in hypothyroid rats of the same strain is  $18.5 \pm 2.1$  hours. Irvine (1967) indicated that exercise increased TSR 38 per cent in the partly trained horses and 65 per cent in the fully trained horses. Concurrently, there was a decrease in protein-bound iodide of 14 and 39 per cent respectively, while the fractional turnover rate of thyroxine increased to 160 and 262 per cent of the resting level. In rats, muscular exercise has been known not to increase the rate of degradation of thyroid hormone, but Escobar del Rey and Morreal de Escobar (1956) found that radiolabeled thyroxine disappeared more quickly in rats when longer periods of

exercise were employed. The study of thyroid function by Rhodes (1967) indicated that there exists a negative correlation between the amounts of exercise and the amount of iodide in the thyroid gland. Oppenheimer and co-workers (1969) showed that increased hepatocellular binding of thyroxine by phenobarbital treatment caused metabolic clearance of thyroxine to augment 61 per cent in which the fractional plasma disappearance rate was increased 34 per cent and the total distribution space gained 19 per cent. The augmented hepatocellular clearance was found to be due to both a 40 per cent increase in deiodinative clearance and an 85 per cent increase in fecal clearance. Direct measurements of biliary clearance indicated that the increased fecal disposition could be attributed exclusively to enhanced biliary excretion. Of particular interest with respect to the biliary studies was the finding that the biliary clearance increased in direct proportion to the increase in the hepatic thyroxine distribution space.<sup>1</sup> The above studies seem to denote that there is a possibility that exercise causes: (i) increased TSR, (ii) increased peripheral utilization, and (iii) increased fractional turnover rate of thyroxine. Based on these assumptions it is possible

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<sup>1</sup>The hepatic thyroxine distribution space is the product of the liver/plasma concentration ratio and the liver weight and can be viewed as an expression of total liver binding.

that, in animals with exogenous L-T<sub>4</sub> as the only source of thyroactive hormone, the increased peripheral utilization of the hormone due to increased muscular activity will decrease the fractional plasma disappearance rate, total distribution space, hepatocellular clearance, and biliary clearance of T<sub>4</sub>, and will cause the concentration of liver cholesterol to increase as it did in groups XX and XVI. This supposition is strengthened when one notes that the simple effect of group XX was much more significant ( $P < .001$ ) than group XVI ( $.02 < P < .05$ ) and groups XVIII and XIX approaching statistical significance ( $.05 < P < .1$ ) when they were compared to their respective NC groups. This may be ascribed to the lower initial circulating level of the daily-administered exogenous L-T<sub>4</sub> in the Hypo-Tdx groups than in the Eu-Tdx groups. The concentration of circulating L-T<sub>4</sub> in group XX may have approached the lower limits of hypothyroidism when exposed to marked physical activity, the half-life of thyroxine being  $18.5 \pm 2.1$  hours (Grossie et al., 1964).

No main effects were found in the Hyper-Tdx ( $0.98 \pm .03$  mg/100 g BW) and Eu-Tdx ( $0.98 \pm .04$  mg/100 g BW) animals when compared to C ( $0.92 \pm .03$  mg/100 g BW). The concentration of circulating L-T<sub>4</sub> in the Hyper-Tdx and Eu-Tdx animals may have been high enough to be effective in allowing normal cholesterologenesis and cholesteroleresis to occur. Eskelson et al., (1970) indicated that various protein binding sites must be

filled, and thus, a threshold concentration of hormone was needed before the hormone elicited an action on HMG-CoA. Moreover, in this study, while the concentration of hepatic total cholesterol in Hyper-Tdx and Eu-Tdx animals was essentially the same as that of C, increased turnover rates in the former must have occurred in the liver in order for the serum hypocholesteremic effect to have ensued in the Hyper-Tdx rats. The increased peripheral utilization of exogenous L-T<sub>4</sub> caused by increased physical activity did not seem to decrease the hepatic cholesterol metabolism in Hyper-Tdx rats. This may be ascribed to the high initial level of administered L-T<sub>4</sub>. No fluctuation in hepatic total cholesterol concentration appeared in groups IX to XII. The increased peripheral utilization of exogenous L-T<sub>4</sub> caused by increased physical activity did lower the rates of hepatic cholesterol metabolism in Eu-Tdx rats in group XVI which exercised to exhaustion. Cholesterol catabolism and excretion decreased, and is reflected by the accumulation of hepatic total cholesterol in group XVI ( $1.07 \pm .04$  mg/100 g BW) as compared to the sedentary Hyper-Tdx group ( $0.90 \pm .03$  mg/100 g BW).

The control rats (with intact thyroids) that were forced to exercise (groups III and IV) demonstrated a tendency to lower serum cholesterol concentration ( $.05 < P < .1$ ). In order that this serum hypocholesteremic response be elicited with the nonsignificant simple effects of the hepatic cholesterol (when compared to group I), the fractional turnover rate

of hepatic cholesterol and bile acid metabolism must have been higher than in the nonexercised controls with intact thyroids. If this supposition is true, then the higher fractional turnover rate elicited by exercise may have been produced by the elevated concentration of circulating endogenous thyroxine. Acknowledging that hepatic cholesterogenesis and cholesteroleresis are dependent on thyroxine and its rates are parallel to the circulating concentration, the mechanism underlying the hypocholesteremic effects of exercise can be postulated (Figure 1, p. 112).

Recent work by a few investigators seem to support this suggested mechanism. Hebbelinck and Casier (1966) and Gollnick and Simmons (1967) demonstrated that forced exercise was effective in increasing the rate of cholesterol catabolism and excretion. They found that the neutral sterol and acidic sterol increased markedly in animals in which exercise was of sufficient intensity and duration. In 1968, Barnard and co-workers conducted an investigation on thyroxine involvement in the reduction of cholesterol associated with chronic exercise. Male Sprague-Dawley rats were classified as trained (1.0 mph daily for 6 weeks on a treadmill) or nontrained and each assigned to four groups: (i) normal, (ii) thyroidectomized, (iii) hypophysectomized, and (iv) hypophysectomized, but receiving thyroid stimulating hormone (TSH). No significant differences of plasma cholesterol was found in either the

trained or nontrained groups. These results suggested that rats must be on a high fat diet before exercise will be associated with a systematic reduction of serum cholesterol levels. In contrast, the hepatic cholesterol in the normal animals was significantly reduced by exercise, whereas the animals devoid of thyroid activity (groups 2 and 3) showed no significant effect due to training. The hypophysectomized rats receiving TSH did exhibit a significant reduction in liver cholesterol. This supports the conjecture that the regulation of cholesterol metabolism is highly dependent on the thyroid gland, and that exercise mediates the serum hypocholesterolemic effect through increased TSR.

Some precaution must be used in interpreting the data of Barnard et al. (1968) since removal of the pituitary removes the source of a host of tropic hormones. This may place the animal in an "unphysiological state", or produce a response to chronic exercise atypical of a normal rat. Nejad and Chaikoff (1963) found that when hypophysectomized rats were given 3 and 6  $\mu\text{g}$  L-T<sub>4</sub>, acetate conversion into hepatic cholesterol increased approximately tenfold over that of the control animals. Beher et al. (1964) demonstrated that the effects of thyroidectomy on steroid metabolism differed from those of hypophysectomy, and it was concluded that the lack of the thyroid hormone in hypophysectomized rats accounted for some, but not all, of the effects of hypophysectomy on steroid

metabolism. Two years later, they demonstrated that while hypophysectomy or thiouracil-hypothyroidism retarded bile acid metabolism by decreasing the end-product turnover rates, the effects of hypophysectomy differed from those of the thiouracil-treated rats (Beher et al., 1966). Moderate thyroid treatment did not restore the steroid turnover or synthesis rates to normal. It was concluded that other factors than the lack of thyroid seemed to have caused the changes in bile acid metabolism effected by hypophysectomy. Moreover, food intake is also known to be altered in hypophysectomized rats. Hahn et al. (1965) reported that hypophysectomy reduced the mean daily feed intake approximately 30 per cent. This is similar to the observation that thyroidectomy-adrenalectomy reduced feed consumption 30 per cent in the investigation of Grossie and Turner (1965). Thus, the reduction in feed intake due to hypophysectomy may also account for the alteration in cholesterol levels, unlike that of thyroidectomized rats.

Despite these short-comings on hypophysectomized-rat studies on steroid metabolism, the possibility that the pituitary and thyroid glands are important in the reduction of serum and hepatic cholesterol, increased fractional turnover rate of cholesterol, and bile acid metabolism is not to be underestimated.



Recently the adrenal glands have been suspected of having a role on the serum cholesterol lowering effect of muscular contractions. Malinow and collaborators (1968a, b, 1969, and 1970) suggested that the adrenal glands may be one of the two major sites at which cholesterol catabolism occurs during increased physical activity. When cholesterol-26- $^{14}\text{C}$  was injected intravenously into an animal, the recovered  $^{14}\text{CO}_2$  in the expired air was used as a quantitative index of cholesterol side-chain degradation. However, their interpretation of the data was misleading when it was implied that cholesterol side-chain degradation in the adrenal glands was considerable. Their cumulative excretion curve indicated that at the end of 80 minutes the measured  $^{14}\text{CO}_2$  accounted for less than one per cent of the total injected labeled cholesterol. During that time interval 80 per cent of the estimated cholesterol oxidation that was dependent on the presence of the adrenals. My calculations indicate that this constituted only 0.1 per cent of the total dose, an amount hardly to be considered an important pathway. Its importance becomes even more questionable when one considers that muscular contractions induced by electrical shocks may be a physiologically different response than that of normal physical activities such as that of running or walking. Here, the problem of "stress" rather than exercise may be evident. It has been long known that cholesterol is a precursor for adrenalcorticoids (Saba and Hechter, 1955). The

electrical shocks may be evoking an increased rate of synthesis and release of adrenal hormones, thereby utilizing the exogenous cholesterol. Their latest study on hepatectomized and adrenalectomized rats (Malinow and associates, 1970) seemed to support their thesis that the adrenals as well as the liver were mainly responsible for splitting the side-chain of cholesterol during rest and during muscular stimulation. Considering the extremely low percentage of cholesterol degradation in reference to the total dose administered, the adrenals contributed 33 to 52 per cent of the oxidative process in the hepatectomized animal. It is possible that the oxidative reaction is direct and rapid in the adrenals, whereas the same process in the liver is under (i) a long-term homeostasis of the endocrine system, (ii) endogenous cholesterol level, and (iii) functional integrity of the entero-hepatic circulation of bile acids. Therefore, the contribution of the liver could have been greater if time was the expanding factor. However, their theory should be considered with caution since there are ample reports on increased adrenal size in rats forced to exercise (Donaldson, 1932, 1933, 1935; McClimtock et al., 1939; Kimeldorf and Baum, 1954; Hearn and Wainio, 1956; Wong et al., 1957; Montoye et al., 1962; Gollnick and Simmons, 1967; McArdle and Montoye, 1967; Tipton et al., 1968; and Ring et al., 1970). This may be an indirect indication that the adrenal activity may be increased during exercise. There

is some evidence that prolonged physical exercise may stimulate the adrenocortical activity in rats (Frenkel and Csalay, 1962), dogs (Suzuki, 1966), and man (Bellet et al., 1969), whereas in other studies no such effect was found (Renold et al., 1951; Hill et al., 1956; Connel et al., 1958; and Papacostas et al., 1960). In none of these studies has there been any indication that the adrenocortical activity, like that of the liver, is thyroxine-dependent for increased cholesterol catabolism. While the reciprocal relationship between the thyroid and adrenocortical response to stress (Harris, 1955), the influence of thyroid hormone on progesterone transformation to C-20 metabolites (Bradlow and co-workers, 1966), the effect of thyroid status on the hydroxylation of estrogen (Fishman et al., 1965), the effect of thyroxine on the conversion of estradiol to estrone (Fishman et al., 1962), and the effects of thyroxine on the alteration of hydrocortisone to 11-ketonic metabolites (Hellman et al., 1961) have been reported, no evidence was submitted on the need for thyroxine in the initial step of the steroidogenic pathway. Simpson and Boyd (1968) indicated that, for tissues in which cholesterol was metabolized to steroid hormones, the first step in the process was the cleavage of the cholesterol side chain to form pregnenolone. This reaction was dependent on NADP and oxygen, and was regulated by ACTH. These evidences suggest that ACTH is the sole regulator of the rate of cholesterol side-chain cleavage,

while ACTH and possibly thyroid hormones affect transformation reactions beyond pregnenolone formation.

In this study the hepatic cholesterol tended to accumulate in the liver of all four A-Tdx groups regardless of the level of physical activity. The serum total cholesterol also was significantly higher than euthyroid controls. Whether exercise stimulated the adrenals directly via a sympathicoadrenal system or by increased ACTH output by the pituitary, the rate of side chain cleavage of cholesterol was apparently not great enough to cause a hypocholesteremic effect in this study. This tends to support the supposition that thyroid activity was necessary for the reduction of serum cholesterol by the liver, and not by the adrenals, when associated with exercise. Further evidence of the unimportance of the adrenal glands in regulating cholesterol metabolism comes from the work of Barnard et al. (1968). They demonstrated a lowering of hepatic cholesterol in exercised control rats, while thyroidectomized and hypophysectomized rats forced to run underwent no change in concentration. However when the hypophysectomized rats were given TSH, hepatic cholesterol reduction occurred in the exercised group. Thus, in the thyroidectomized animals, where ACTH or glucocorticoids were potentially available via exercise, no change in hepatic total cholesterol was found. Hypophysectomized rats given TSH and forced to exercise (with no available source of endogenous ACTH) underwent a reduction.

Data from our study on adrenal weights (Table 7) suggested that exercise did not induce adrenal hypertrophy in any of the groups when main effects were compared. This tends to imply that adrenal weights were independent of the level of physical activity and thyroid activity.

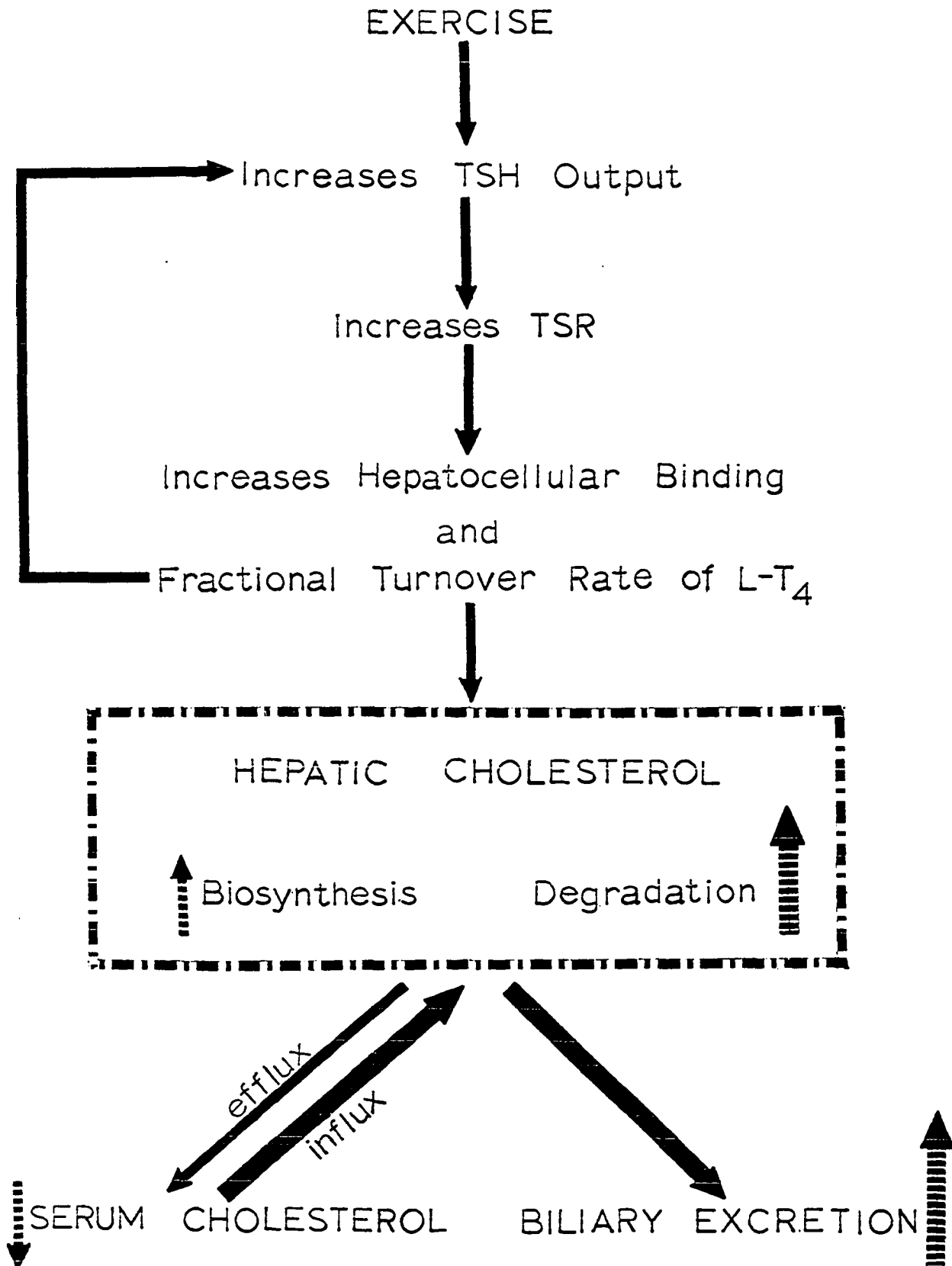
Therefore, it can be concluded that the mechanism by which serum total cholesterol is lowered in rats forced to exercise exhaustively is mediated indirectly by increased thyroid activity, which in turn is stimulated by increased release of TSH from the anterior pituitary gland. Exercise may increase TSH release by two possible pathways: (i) by directly stimulating the hypothalamus to release TRF (thyroid releasing factor) via the CNS or (ii) by increasing the peripheral utilization and fractional turnover rate of thyroxine, reducing the negative feedback on the hypothalamus or adenohypophysis so that TSH is released. Subsequently, hepatocellular binding and fractional turnover rate of thyroxine are increased, allowing hepatic cholesterol degradation and excretion rates to exceed the biosynthetic rate. This causes the serum cholesterol influx into the liver to transcend the rate of efflux so that, ultimately, the cholesterol pool in the serum is lowered. Moreover, this catabolic process occurs mainly in the thyroxine-dependent liver and not in the adrenal glands.

This proposed mechanism does not mean that the thyroid hormone alone regulates cholesterol metabolism. There is a possibility that other hormones contribute to the hypocholesteremic effect. Recently Friedman et al. (1970) demonstrated that growth hormone was as effective as the thyroid extract in reducing the postoperative hypercholesterolemia of the hypophysectomized rats. Neither hormones alone could completely inhibit the development of the rising cholesterol levels in the hypophysectomized rats. When the two hormones were given in combination, hypercholesterolemia was completely prevented. A serum cholesterol concentration resulted which was similar to that of the control rats with intact thyroid and pituitary glands.

Moreover, other metabolic effects not mediated by hormones could have been a contributing factor in lowering serum cholesterol levels in exercised rats. The possible reduced levels of acetyl-CoA, NADPH, ATP and other metabolic co-factors due to increased physical activity, could have depressed the rate of cholesterologenesis in all tissues. This would have led to a lower turnover rate of cholesterol between the tissue and serum cholesterol pools, resulting in a lower serum total cholesterol concentration.

Figure 1. Suggested mechanism by which hypocholesteremia is mediated by increased physical activity

Exercise may increase the rate of release of TRF (thyrotropic releasing factor) by the hypothalamus which in turn accelerates the release of TSH (thyroid stimulating hormone) from the anterior pituitary gland. Exercise may (i) directly stimulate the hypothalamus to release TRF via the CNS (central nervous system) or (ii) exercise may increase the peripheral utilization of thyroxine which inhibits the negative feedback on the anterior hypothalamus or adenohypophysis to release TSH. The increasing level of TSH generates the thyroid into increasing the rate of synthesis and release of thyroxine (TSR) into the blood. Subsequently, hepatocellular binding and fractional turnover rate of thyroxine are increased. This allows the hepatic cholesterol degradation and excretion rates to exceed the biosynthetic rate. This causes the serum cholesterol influx into the liver to transcend the rate of efflux which ultimately lowers the cholesterol pool in the serum.





## SUMMARY

A 4 x 5 factorial investigation was conducted on the effects of four levels of physical activity and five thyroid levels on serum and hepatic total cholesterol concentrations.

Subcutaneous administration of L-T<sub>4</sub> (Na-salt, L-thyroxine) in physiological amounts to 70-day old thyroidectomized rats was performed daily for 10 weeks. The following five thyroid states were produced: (i) athyroidism, no L-T<sub>4</sub> replacement therapy; (ii) hyperthyroidism, 3.5 µg L-T<sub>4</sub>/100 g BW; (iii) euthyroidism, 1.0 µg L-T<sub>4</sub>/100 g BW; (iv) hypothyroidism, 0.5 µg L-T<sub>4</sub>/100 g BW. The fifth group, rats with intact thyroid glands, served as (v) euthyroid controls.

Each of the above five levels of thyroid activity were further subdivided into four levels of physical activity: (i) nonexercised controls, (ii) standing in three inches of water for 15 minutes to check the possibility of induced stress of water, (iii) moderately exercised, and (iv) exhaustively exercised. In the latter two groups, the rats were individually swum in 40 gallon plastic tanks six days a week for 10 weeks. The exhaustively exercised groups were swum until they could no longer remain swimming above water (10 seconds submergence). The moderate exercise was arbitrarily established as one-half the mean weekly swimming time of the respective exhaustively exercised groups. To decrease the swimming time and to

stimulate more vigorous swimming, lead weights (4 per cent of BW) were attached to the tail of both forced-exercised groups.

Each of the 20 treatment combinations contained 12 male rats. Both treatments (thyroxine replacement and exercise) began on day 70 and lasted for 10 weeks. The influence of feed consumption on the cholesterol concentrations was ascertained by measuring the daily feed intake. The following results were obtained:

1. The lack of L-T<sub>4</sub> (as in the athyroid rats) and physiologically-induced hyperthyroid animals had lower final body weights than the euthyroid control rats. It appeared that the reduction in final body weight of the athyroid rats was due to the stunted growth and the lack of epididymal fat depots. In the hyperthyroid rats the latter phenomenon was more evident.

2. The final body weight in both the moderately and exhaustively exercised rats was significantly lower than that of the sedentary controls. The reduction in body weight was greater in the latter group, suggesting that a direct relationship between the severity of exercise and weight reduction may exist. Specifically, the lower final body weight (as compared to the sedentary control) in the exercised animals was found in the C, Eu-Tdx, and Hypo-Tdx rats. They seemed to possess normal or excess amounts of epididymal fat depots. In contrast, the A-Tdx and Hyper-Tdx rats with little fat depots, which may be representing the fat-depleted tissues, offered little

opportunity for weight reduction during the exercise regimen.

The lower final body weight in rats that swam to exhaustion was also due, in part, to the lower daily feed intake.

3. A significant direct relationship between thyroid status and consumption of food was found.

4. The averaged means of daily feed intake was the same in the moderately exercised and nonexercised groups. In contrast, the exhaustively exercised rats had an over-all lower daily feed consumption as compared to the nonexercised controls. An apparent indirect relationship existed between increasing physical activity and feed intake.

5. There appeared to be an inverse relationship between serum total cholesterol levels and thyroid hormone concentration.

The main effect noted in the A-Tdx, Eu-Tdx, and Hypo-Tdx groups (when compared to C) was that the serum total cholesterol concentration was considerably higher than in C. The higher serum total cholesterol level in the Hypo-Tdx groups and particularly in the Eu-Tdx groups, may have been due to the decreasing concentration of the daily-administered-exogenous L-T<sub>4</sub> which may be attributable to the continued peripheral utilization of the L-T<sub>4</sub>. This may have rendered the liver ineffective in controlling the normal metabolism of cholesteroleresis. The Hyper-Tdx animals were not affected by the continued peripheral utilization of L-T<sub>4</sub> as in the Eu-Tdx and

Hypo-Tdx rats. It appeared that while, peripheral utilization occurred in the Hyper-Tdx animals, as in other animals, the decrease in concentration of the exogenously-administered L-T<sub>4</sub> was not great enough to decrease the efficiency of cholesteroleresis. This may possibly be ascribed to the high initial daily dose of L-T<sub>4</sub> (3.5 µg/100 g BW).

6. Physical activity had no over-all effect on the lowering of serum cholesterol in those animals without intact thyroids, regardless of the existing thyroid status with which they were associated. The control animals with intact thyroid glands had a tendency to lower their serum cholesterol concentration when forced to exercise. This suggested increased thyroid activity as a possible explanation for the serum hypocholesteremic effect of exercise.

7. Hepatic total cholesterol was found to accumulate in all A-Tdx groups regardless of the severity of physical activity. Since no L-T<sub>4</sub> was present in these animals, the accumulation of hepatic total cholesterol suggested the possibility that the liver was dependent on thyroxine for normal cholesteroleresis. There seemed to be a threshold concentration of thyroxine that was necessary for normal hepatic function. Levels below this threshold concentration rendered the liver ineffective in maintaining normal cholesteroleresis. This phenomenon was observed in the thyroidectomized rats given replacement therapy of L-T<sub>4</sub> to establish a hyper-, eu-,

and hypothyroid states. There was a direct correlation between the amount of daily-administered-exogenous L-T<sub>4</sub> and the amount of physical activity that must be performed before hepatic total cholesterol accumulated. In the Hypo-Tdx rats moderate exercise caused a tendency for hepatic cholesterol to accumulate; and exhaustive exercise produced a greater accumulation of hepatic cholesterol. Hepatic total cholesterol accumulated only in the exhaustively exercised Eu-Tdx group, while no fluctuation of hepatic cholesterol levels occurred in the Hyper-Tdx animals. These results suggested that (i) increased peripheral utilization of thyroxine occurred with increased activity and, (ii) in order for the thyroxine-dependent liver to be effective in controlling normal cholesteroleresis, the circulating level of thyroxine must be above a certain threshold concentration.

While no apparent change in the hepatic total cholesterol concentration appeared in the moderately and exhaustively exercised euthyroid control animals (with intact thyroid), the hepatic cholesterol and bile acid turnover rates must have been augmented in order for the serum total cholesterol level to tend to decline with increased physical activity. This indicates that exercise may directly or indirectly increase thyroidal activity.

8. The serum hypocholesterolemic effect of exercise appeared to be independent of feed intake and is regulated primarily by the thyroxine-dependent liver rather than by the adrenal glands.

## ADDENDUM TO TRIGLYCERIDE STUDY

Although cholesterol ester is the major class of lipid that accumulates in atheromatous lesion (Bottcher and Woodford, 1962), triglycerides, phosphoglycerides, sphingolipids, and free cholesterol are present in smaller quantities. According to the Framingham study (Kannel, 1966), it was found that plasma cholesterol and triglyceride levels were positively correlated with coronary artery disease. Although serum cholesterol concentration has been widely utilized as a diagnostic and prognostic indicator for atherosclerosis and other vascular degenerative diseases, there are increasing reports that triglycerides (TG) may be as important.

In 1964, Holloszy et al. were the first to report that exercise had a significant effect on lowering serum triglyceride. Serum total cholesterol and phospholipid levels were not affected by the six-month exercise program. Similar reports were made by a Swedish group on men (Carlson and Mossfeldt, 1964; Carlson and Fröberg, 1967; and Carlson and co-workers, 1967) and on rats (Carlson and Fröberg, 1969).

An extensive study by Bassett and collaborators (1969a, b, c) on serum lipids, cardiovascular disease, anthropometric and related parameters in Japanese and Hawaiian men of Oahu, Hawaii, indicated that abnormalities in energy balance rather than differences in specific nutrient intake, appeared to

account for the differences in the frequency of coronary heart disease. Analysis of nutrient intake showed that the saturated-polyunsaturated fatty acid ratio and cholesterol were significantly higher in Hawaiians in both the hospital controls and the case patients (with a history of myocardial infarction). If these dietary factors were to exert their major pathogenic influence via the production of hypercholesterolemia, one would expect to find the average serum cholesterol of the Hawaiians to be higher than that of the Japanese. Such was not the case. Hawaiian men tended to have somewhat lower serum cholesterol levels than did the Japanese men. Examination of the work history-to-physical activity-index showed that Hawaiian cases and hospital controls had a significantly higher work history index than did their Japanese counterparts. According to this index, Hawaiians engaged in heavier manual work over their working lives and presumably expended more physical energy in that work than did the Japanese. Based on the current average 24-hour energy expenditure at the time of the investigation, the Hawaiians and Japanese did not differ significantly in physical activity index. An earlier study by Bennett et al. (1962) indicated that the Hawaiians had the highest mortality rate from coronary heart disease of any ethnic group in Hawaii, in striking contrast to the low mortality rate found among the Japanese in Hawaii. There was some evidence from their study, but only of a suggestive nature, that the Hawaiians as a group



had somewhat higher triglyceride concentrations than the Japanese. Moreover, the finding of hyperglyceridemia (fasting triglycerides above 150 mg per 100 cc) was greater in Hawaiian cases and hospital controls than in Hawaiian population controls (41 and 38 vs 24 per cent) but not in their respective Japanese counterparts (25 and 25 vs 25 per cent). This suggested that TG may be a better prognostic indicator for potential heart attack subjects.

Recently Karvonen and co-workers (1970) conducted a five-year study on the incidence of CHD in Finland and found that, while the inhabitants were notably lean and physically active by American standards, the serum cholesterol levels were very high and the incidence of CHD was the highest of all European countries. While statistically the incidence of CHD was not related to physical activity (nor to body weight or to cigarette smoking) the data did suggest that the incidence was excessive among moderately active men, but the extremely active men were less prone to the disease. Unfortunately no other lipid fractions of the serum were analyzed.

In view of these recent findings, a brief discussion of the influence of physical activity and thyroid activity on serum triglycerides will be presented from the present investigation.

Estimation of serum TG values were made from the same sera samples provided by the cholesterol study. The analyses were conducted by the modification of Kessler and Leaderer's method (1966) by substituting silicic acid for zeolite (Ewan, 1969). The data were tabulated and analyzed by Iowa State University, Statistical Laboratory, Ames, Iowa, by employing analysis of variance and analysis of covariance to the 20 treatment combinations under the 4 x 5 factorial experimental design. The results are presented in Table 8.

The results of the analysis of variance indicated that thyroid status (A), physical activity (B), and AB interaction had a highly significant influence ( $P < .0005$ ) on the serum triglyceride level.

When the 20 treatment combination means were adjusted to account for the varying feed intake by the use of analysis of covariance, factor A ( $P < .0005$ ), factor B ( $P < .0005$ ) and AB interaction ( $P < .01$ ) were found to be significant. This indicated that feed intake was not an important factor in influencing the serum TG concentrations when the rats were fed a basal diet. This also suggested that the serum TG levels represent primarily the endogenous TG. The unadjusted means were used when determining the effects of the thyroid status and physical exercise on serum TG levels.

When compared to C, the main effect of A-Tdx, Hyper-Tdx, Eu-Tdx, and Hypo-Tdx was found to be significantly lower

(P < .0001).

The lower serum TG level in the A-Tdx and Hypo-Tdx animals could be attributed to several factors. Dayton et al. (1960) have shown that acetate (a key intermediary compound in both the de novo synthesis and catabolism of fatty acids) turnover rate is largely proportional to the metabolic rate of the animal. In hyperthyroidism the turnover rate of fatty acids is elevated and in hypothyroidism the turnover rate is decreased. Lipsky et al. (1955) have demonstrated a striking decrease in the incorporation of acetate-1-<sup>14</sup>C into plasma TG in patients with myxedema and a return to normal of lipid synthesis from acetate-1-<sup>14</sup>C following triiodothyronine (T<sub>3</sub>) administration. The precise mechanism by which the lack of thyroactive compounds decrease the rate of endogenous TG synthesis is not known. It has been suggested that the low concentrations of several co-factors (i.e., coenzyme A, ATP, TPNH) in hypothyroid animals may provide an explanation to the decreased synthetic rates. These co-factors are necessary for optimum lipogenesis (Tabachnick and Bonnycastle, 1954; Glock and McLean, 1955; and Bronk and Bronk, 1962). The possible lower de novo fatty acid and TG synthesis in athyroid and hypothyroid animals due to the lowered concentration of these co-factors could ultimately explain the lower serum TG concentration that was found in this investigation. The low amounts of epididymal fat depots in the A-Tdx animals gives supportive evidence to

the conjecture that de novo fatty acid and TG synthesis is depressed in animals with lower levels of circulating thyroxine.

An alternate explanation to the lower serum TG level in the A-Tdx and Hypo-Tdx animals (compared to C) could be ascribed to the lower or impaired mobilization of free fatty acids (FFA) to the liver for TG synthesis. It has been shown that the lipolytic action of the catecholamines, ACTH, and growth hormone is greatly dependent upon adequate thyroid function (Pothier and Italie, 1960; Hamburger et al., 1961; and Debons and Schwartz, 1961). Dayton et al. (1960) has shown that the hypothyroid rat has a lower rate of hepatic TG synthesis. Thus, the lower circulating  $T_4$  concentration in the A-Tdx and Hypo-Tdx rats in this study could be a factor in the possible impaired FFA mobilization from the adipose tissue to the liver for TG synthesis.

The Eu-Tdx animals also had a significantly lower mean concentration of serum TG when compared to C. This could possibly be attributed to the lower level of circulating exogenous  $T_4$  due to the continued peripheral utilization by the tissues. The cholesterol study indicated that it is highly probable that the Eu-Tdx rats were in a physiologically hypothyroid state. If this supposition is true, then the lower serum TG concentration found in the Eu-Tdx animals is explainable by the above discussion on A-Tdx and Hypo-Tdx rats.

The rats in the Hyper-Tdx groups also had significantly lower serum TG (compared to C). This is in agreement with O'Hara et al. (1966) who demonstrated that thyroxine administration to myxedematous patients results in a decline in serum TG. It has been suggested by Porte et al. (1966) that the lowered serum TG levels due to thyroxine administration may be due to increased postheparin lipolytic activity and tissue lipoprotein lipase activity. These enzymes causes hydrolysis of serum TG and possible incorporation into the tissues for utilization as fuel to meet the higher metabolic demand.

The notable leanness in the Hyper-Tdx rats (as compared to C, Eu-Tdx, and Hypo-Tdx) may be an indication that:

(i) increased TG lypolysis in the adipocytes occurred through the elevated hormone-sensitive lipase activity, (ii) resulting in FFA mobilization to the peripheral tissues for preferential fuel. This would (iii) decrease the available FFA mobilization to the liver for TG synthesis which (iv) would result in a lower rate of release of the newly synthesized TG into the blood. This could account for the lower serum TG levels in the Hyper-Tdx rats in this study.

The serum TG levels were considerably lower (main effect) in the moderately exercised groups ( $.02 < P < .05$ ) and exhaustively exercised groups ( $P < .001$ ) when compared to NC. This is in agreement with Holloszy et al. (1964), Carlson and

Mossfeldt (1964), Carlson and Fröberg (1967, 1969), Carlson et al. (1967), Jones and Havel (1967), and Papadopoulos et al. (1969). The results of this investigation indicate that (i) exercise is effective in lowering serum TG and (ii) this hypotriglyceridaemic response is directly related to the increasing severity of exercise.

The mechanism for this change is not clearly understood. It is possible that the cause of the decreased plasma TG during exercise may be due to a decreased TG efflux into the plasma. Since a large portion of the endogenous plasma TG are derived from the liver (Masoro, 1968), a decreased efflux of TG to plasma could be caused by a decreased delivery of endogenous TG hepatic the plasma. This might occur as a result of decreased hepatic uptake of plasma FFA (Havel and Goldfien, 1961). While the mobilization of FFA from adipose tissue is actually increased (Friedberg et al., 1963; Pruett, 1970), plasma FFA uptake by the muscles is also increased (Carlson and Pernow, 1959, 1961; Havel et al., 1967). The increased plasma FFA uptake by the muscles could be the result of (i) FFA as the preferential fuel for muscles during the elevated metabolic demand of exercise (Åstrand and Rodahl, 1970) or (ii) a change in the distribution of cardiac output during exercise. It has been shown that blood flow through the working muscles is increased (Carlsten and Grimby, 1966) while hepatic blood flow is considerably reduced during heavy exercise (Wade and Bishop,

1962). This suggests that the uptake of plasma FFA by the liver may be decreased during exercise, which could result in a decreased formation of TG in the liver. The rapid equilibration between the hepatic and plasma TG pools seen in rabbits (Havel et al., 1962) and in man (Carlson and Ekelund, 1963) makes it probable that a decrease of the hepatic TG pools could be reflected in a decrease of the plasma TG pools.

Another possible mechanism by which hypotriglyceridaemia occurs as a result of increased physical activity may be due to the increased influx of TG from the plasma into tissues. Nikkilä et al. (1963) reported that lipoprotein lipase activity was increased in rats forced to exercise. The increased lipase activity increased the substrates (FFA hydrolyzed from beta-lipoproteins) available to the working muscles for fuel. The increased lipoprotein lipase activity may be the result of increased TSR due to exercise. Porte et al. (1966) reported that lipoprotein lipase activity is increased after thyroxine therapy. Thus, it is possible that hypotriglyceridaemia due to exercise may be mediated indirectly through the thyroid gland as was demonstrated in the rats with intact thyroids in this investigation (groups I-IV).

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## BIBLIOGRAPHY

- Albrink, M. J. 1965. Diet and cardiovascular disease. J. Amer. Dietetic Ass. 46:26.
- Ahrens, R. A. and Broxton, M. H. 1970. The effects of various physical activity levels and dietary carbohydrate source on rat cholesterol levels. Proc. Soc. Exp. Biol. Med. 134:1043.
- Anderson, J. T. 1952. Changes in blood cholesterol and lipoproteins during experimental overnutrition. Fed. Proc. 11:181.
- Anderson, T. A., Geber, W. F., Duick, D., Pickard, T. and Dyne, B. Van. 1965. Audio-visual stress and blood serum protein in the cholesterol-fed rat. Exp. Med. Surg. 23:375.
- Anderson, Z. E. 1959. Our industry today: current dietary recommendations for the prevention and treatment of coronary heart disease and what they mean to the dairy industry. J. Dairy Sci. 42:1401.
- Anfinsen, C. B. and Horning, M. G. 1953. Enzymatic degradation of the cholesterol side chain in cell-free preparations. J. Amer. Chem. Soc. 75:1511.
- Anthony, A. and Babcock, S. 1958. Effects of intense noise on adrenal and plasma cholesterol of mice. Experientia 14:104.
- <sup>O</sup>Astrand, P. and Rodahl, K. 1970. Textbook of work physiology. McGraw-Hill Book Co., New York.
- Baker, M. A. and Horvath, S. M. 1964. Influence of water temperature on oxygen uptake of swimming rats. J. Appl. Physiol. 19:1215.
- Balke, B. and Ware, R. 1959. An experimental study of "physical fitness" of Air Force personnel. U.S. Armed Forces Med. J. 10:675.
- Barboriak, J. J., Kory, R. C., Hamilton, L. H. and Kelley, F. P. 1966. Changes in breakfast menu and blood lipids. J. Amer. Dietetic Ass. 49:204.

- Barnard, R. J., Terjung, R. L. and Tipton, C. M. 1968. Hormonal involvement in the reduction of cholesterol associated with chronic exercise. *Int. Z. angew. Physiol. einschl. Arbeitsphysiol.* 25:303.
- Bassett, D. R., Abel, M., Moellering, R. C., Jr., Rosenblatt, G. and Stokes, J. 1969a. Coronary heart disease in Hawaii: Dietary intake, depot fat, "stress," smoking, and energy balance in Hawaiian and Japanese men. *Amer. J. Clin. Chem.* 22:1483.
- Bassett, D. R., Abel, M., Moellering, R. C., Jr., Rosenblatt, G. and Stokes, J. 1969b. Dietary intake, smoking history, energy balance, and "stress" in relation to age and to coronary heart disease risk in Hawaiian and Japanese men in Hawaii. *Amer. J. Clin. Nut.* 22:1504.
- Bassett, D. R., Moellering, R. C., Jr., Rosenblatt, G., Greenberg, D. and Stokes, J. 1969c. Coronary heart disease in Hawaii. Serum lipids, and cardiovascular, anthropometric, and related findings in Japanese and Hawaiian men. *J. Chron. Dis.* 21:565.
- Baumann, E. J. and Holly, O. M. 1926. Cholesterol and phosphatide metabolism in pregnancy. *Amer. J. Physiol.* 75:618.
- Beck, H., Simpson, M. E., Evans, H., Ray, R. D., Li, C. H. and Asling, C. W. 1946. Response to pituitary growth hormone and thyroxin of the tibias of hypophysectomized rats after long postoperative intervals. *Anat. Rec.* 94:631.
- Behr, W. T. and Baker, G. D. 1959. Build-up and regression of inhibitory effects of cholic acid on in vivo liver cholesterol synthesis. *Proc. Soc. Exp. Biol. Med.* 101:214.
- Behr, W. T., Baker, G. D. and Anthony, W. L. 1959. Effect of dietary cholic acid on in vivo cholesterol metabolism. *Proc. Soc. Exp. Biol. Med.* 100:3.
- Behr, W. T., Baker, G. D. and Penney, D. G. 1963. A comparative study of the effects of bile acids and cholesterol in cholesterol metabolism in the mouse, rat, hamster, and guinea pig. *J. Nut.* 79:523.

- Beher, W. T., Baker, G. D., Beher, M. E., Vulpetti, A. and Semenuk, G. 1964. Effects of hypophysectomy, thyroidectomy and thyroid hormones on steroid metabolism in the rat. *Proc. Soc. Exp. Biol. Med.* 117:738.
- Beher, W. T., Beher, M. E. and Semenuk, G. 1966. The effect of pituitary and thyroid hormones on bile acid metabolism in the rat. *Metabolism* 15:181.
- Beher, W. T., Casazza, K. K., Beher, M. E., Filus, A. M. and Bertasius, J. 1970. Effects of cholesterol on bile acid metabolism in the rat. *Proc. Soc. Exp. Biol. Med.* 134:595.
- Belle, H. Van. 1965. Cholesterol, bile acids, and atherosclerosis. North-Holland Pub. Co., Amsterdam.
- Bellet, S., Roman, L. and Barham, F. 1969. Effects of physical exercise on adrenocortical excretion. *Metabolism* 18:484.
- Bennett, C. G., Tokuyama, G. H. and McBride, T. C. 1962. Cardiovascular-renal mortality in Hawaii. *Amer. J. Public Health* 52:1418.
- Bergström, S., Danielsson, H. and Samuelsson, B. 1960. Formation and metabolism of bile acids. In Bloch, K., Ed. *Lipid metabolism*. Pp. 291-336. John Wiley and Sons, Inc., New York, N.Y.
- Bergström, S. and Norman, A. 1953. Metabolic products of cholesterol in bile and feces of rat. *Steroids and bile acids. Proc. Soc. Exp. Biol. Med.* 83:71.
- Bergström, S. and Sjövall, J. 1954. Occurrence and metabolism of chenodesoxycholic acid in the rat. *Bile acids and steroids* 13. *Acta Chem. Scand.* 8(1):611.
- Berkson, D. M. and Stamler, J. 1965. Epidemiological findings on cerebrovascular diseases and their implications. *J. Atheroscler. Res.* 5:189.
- Bernick, S. and Patek, P. R. 1961. Effect of cholesterol feeding on morphology of selected endocrine glands. *Arch. Pathol.* 72:321.
- Berseus, O. and Danielsson, H. 1963. On the metabolism of coprostan-3 $\alpha$ ,7 $\alpha$ -diol in mouse liver homogenates. *Bile acids and steroids* 130. *Acta Chem. Scand.* 17:1293.

- Best, M. M. and Duncan, C. H. 1956. Effects of sitosterol on cholesterol concentration in serum and liver in hypothyroidism. *Circulation* 14:344.
- Best, M. M. and Duncan, C. H. 1959. Effect of thiouracil and sitosterol in diet-induced hypercholesterolemia and lipomatous arterial lesions in the rat. *Amer. Heart J.* 58:214.
- Best, M. M. and Duncan, C. H. 1960. Effect of certain thyroxine analogues on liver cholesterol. *Amer. J. Physiol.* 199:1000.
- Beveridge, J.M.R., Connell, W. F. and Mayer, G. A. 1956. Dietary factors affecting the level of plasma cholesterol in humans: The role of fats. *Canad. J. Biochem.* 34:441.
- Bloch, K. 1954. Biological synthesis of cholesterol. *Harvey Lecture Series* 48:68.
- Bloch, K. 1957. The biological synthesis of cholesterol. *Vitamins and Hormones* 15:119.
- Bloch, K., ed. 1960. *Lipid metabolism*. John Wiley and Sons, Inc., New York, N.Y.
- Bloch, K. 1965. The biological synthesis of cholesterol. *Science* 150:19.
- Bloch, K. and Rittenberg, D. 1942. On the utilization of acetic acid for cholesterol formation. *J. Biol. Chem.* 145:625.
- Bloch, K., Berg, B. N. and Rittenberg, D. 1943. The biological conversion of cholesterol to cholic acid. *J. Biol. Chem.* 149:511.
- Bloomfield, D. K. 1963. Dynamics of cholesterol metabolism I. Factors regulating total sterol biosynthesis and accumulation in the rat. *Proc. Nat. Acad. Sci.* 50:117.
- Blumgart, H. L., Freedberg, H. S. and Kurland, C. S. 1950. Hypothyroidism produced by radioactive iodine ( $I^{131}$ ) in treatment of euthyroid patients with angina pectoris and congestive heart failure. *Circulation* 1:1105.
- Borrow, J. G. 1960. Level of cholesterol differences among monks. *Ann. Intern. Med.* 52:368.

- Bottcher, C. J. and Woodford, F. P. 1962. Chemical changes associated with atherosclerosis. *Fed. Proc.* 21(4):15.
- Boyd, E. M. 1936. The effects of thyroidectomy on blood lipids. *Trans. Royal. Soc. Canad.* 30(5):11.
- Boyd, E. M. and Connell, W. F. 1936. Thyroid disease and blood lipids. *Quart. J. Med.* 29:455.
- Boyd, G. S. 1959. Thyroid function, thyroxine analogs, and cholesterol metabolism in rats and rabbits. In Pincus, G., ed. *Hormones and atherosclerosis*. Pp. 49-62. Academic Press, New York, N.Y.
- Boyd, G. S. 1961. Endocrines in lipid metabolism. *Fed. Proc.* 20:152.
- Boyd, G. S. and Oliver, M. F. 1960a. Various effects of thyroxine analogues on the heart and serum cholesterol in the rat. *J. Endocrinology* 21:35.
- Boyd, G. S. and Oliver, M. F. 1960b. Thyroid hormones and plasma lipids. *Brit. Med. Bull.* 16:138.
- Bradlow, H. L., Fukushima, D. K., Zumoff, B., Hellman, L. and Gallagher, T. F. 1966. Influence of thyroid hormone on progesterone transformation in man. *J. Clin. Endocrinol. Metab.* 26:831.
- Brainard, J. B. 1959. Effect of prolonged exercise on atherogenesis in the rabbit. *Proc. Soc. Exp. Biol. Med.* 100:244.
- Breslow, L. and Buell, P. 1960. Mortality from coronary heart disease and physical activity of work in California. *J. Chron. Dis.* 11:421.
- Brewer, W. D. and Arnrich, L. 1958. The role of fat in the diet. *J. Home Ec.* 50:269.
- Bronk, J. R. and Bronk, M. S. 1962. Influence of thyroxine on oxidative phosphorylation in mitochondria from thyroidectomized rats. *J. Biol. Chem.* 237:897.
- Bronstein, I. P. 1933. Studies in cretinism and hypothyroidism in childhood. I. Blood cholesterol. *J. Amer. Med. Ass.* 100:1661.

- Brown, C. E., Huang, T. C., Bortz, E. L. and McCay, C. M. 1956. Observations on blood vessels and exercise. *J. Gerontology* 11:292.
- Bruggen, J. T. Van, Hutchens, T. T., Claycomb, C. K., Cathey, W. J. and West, E. S. 1952. The effect of fasting upon lipogenesis in the intact rat. *J. Biol. Chem.* 196:389.
- Brumbach, W. B. 1961. Changes in the serum cholesterol levels of male college students who participated in a special physical exercise program. *Res. Quart.* 32(2):147.
- Brunner, D., Loebl, K. and Altman, S. 1962a. Influences of manual labor on lipid values and their relation to the incidence of coronary artery disease. *Circulation* 26:693 (abstr.).
- Brunner, D., Manelis, G. and Loebl, K. 1962b. Influence of manual labor and occupation on the five-year mortality of middle-aged patients with initial myocardial infarctions. *Circulation* 26:693 (abstr.).
- Bucher, N.L.R., McGrrahan, K. and Gould, E. 1959. Cholesterol biosynthesis in preparations of liver from normal, fasting, X-irradiated, cholesterol-fed, triton, or  $\Delta^4$ -cholesten-3-one-treated rats. *J. Biol. Chem.* 234:262.
- Bucher, N.L.R., Overath, P. and Lynen, F. 1960.  $\beta$ -hydroxy- $\beta$ -methyl-glutaryl coenzyme A reductase, cleavage and condensing enzymes in relation to cholesterol formation in rat liver. *Biochim. Biophys. Acta* 40:491.
- Byers, S. O. 1958. The mechanism for changes in blood cholesterol in deranged thyroid states. *Amer. J. Clin. Nut.* 6:642.
- Byers, S. O., Rosenman, R. H., Friedman, M. and Biggs, M. W. 1952. Rate of cholesterol synthesis in hypo- and hyperthyroid rats. *J. Exp. Med.* 96:513.
- Calvy, G. L., Cady, L. D., Mufson, M. A., Nierman, J. and Gertler, M. M. 1963. Serum lipids and enzymes. *J. Amer. Med. Ass.* 183:1.
- Calvy, G. L., Coffin, L. H., Gertler, M. M. and Cady, L. D. 1964. The effect of strenuous exercise on serum lipids and enzymes. *Military Med.* 129:1012.

- Campbell, D. E. 1965. Influence of several physical activities on serum cholesterol concentrations in young men. *J. Lipid Res.* 6:478.
- Campbell, D. E. 1966. Influence of diet and physical activity on blood serum cholesterol of young adult males. *Amer. J. Clin. Nut.* 18:79.
- Campbell, D. E. 1967. Effect of controlled running on serum cholesterol of young adult males of varying morphological constitutions. *Res. Quart.* 39:47.
- Carlson, L. A. 1967. Lipid metabolism and muscular work. *Fed. Proc.* 26(6):1755.
- Carlson, L. A. and Fröberg, S. O. 1967. Blood lipid and glucose levels during a ten-day period of low-calorie intake and exercise in man. *Metabolism* 16:624.
- Carlson, L. A. and Ekelund, L. G. 1963. Splanchnic production and uptake of endogenous triglycerides in the fasting state in man. *J. Clin. Invest.* 42:714.
- Carlson, L. A. and Fröberg, S. O. 1969. Effects of training with exercise on plasma and tissue lipid levels of aging rats. *Gerontologia* 15:14.
- Carlson, L. A. and Mossfeldt, F. 1964. Acute effects of prolonged, heavy exercise in the concentration of plasma lipids and lipoproteins in man. *Acta Physiol. Scand.* 62:51.
- Carlson, L. A. and Pernow, B. 1959. Studies on blood lipids during exercise. I. Arterial and venous plasma concentration of unesterified fatty acids. *J. Lab. Clin. Med.* 53:833.
- Carlson, L. A. and Pernow, B. 1961. Studies on blood lipids during exercise. II. The arterial plasma free fatty acid concentration during and after exercise and its regulation. *J. Lab. Clin. Med.* 58:671.
- Carlson, L. A., Ekelund, L. G., Fröberg, S. O. and Hallberg, D. 1967. Effect of exercise on the elimination of exogenous triglycerides from blood in man. *Excerptum, Acta Med. Scand.*, 182 Suppl. 472:245.
- Carlson, L. A., Fröberg, S. O. and Nye, E. R. 1968. Effect of age on blood and tissue lipids levels in the male rat. *Gerontologia* 14:65.

- Carlsten, A. and Grimby, G. 1966. The circulatory response to muscular exercise in man. Charles C. Thomas, Pub., Springfield, Ill.
- Chaikoff, I. L., Enteman, C., Changus, G. W. and Reichert, F. L. 1941. Influence of thyroidectomy on blood lipids of the dog. *Endocrinology* 28:797.
- Chaikoff, I. M., Siperstein, M. D., Dauben, W. G., Bradlow, H. L., Eastham, J. F., Tomkins, G. M., Meier, J. R., Chen, R. W., Hotta, S. and Spere, P. A. 1952. C<sup>14</sup>-Cholesterol: II. Oxidation of carbons 4 and 26 to carbon dioxide by the intact rat. *J. Biol. Chem.* 194:413.
- Chanutin, A., Gjessing, E. C. and Ludewig, S. 1947. Effect of alpha-naphthylthurea (ANTU) on serum cholesterol in thyroidectomized dogs. *Proc. Soc. Exp. Biol. Med.* 64:174.
- Chapman, J. M. and Massey, F. J. 1964. The inter-relationship of serum cholesterol, hypertension, body weight and risk of coronary disease; results during ten-years follow-up in the Los Angeles heart study. *J. Chron. Dis.* 17:933.
- Chapman, J. M., Reeder, L. G., Massey, Jr., F. J., Borun, E. R., Picken, B., Browning, G. G., Coulson, A. H. and Zimmerman, D. H. 1966. Relationships of stress, tranquilizers, and serum cholesterol levels in a sample population under study for coronary heart disease. *Amer. J. Epidemiol.* 83:537.
- Chevallier, F. 1967. Dynamics of cholesterol in rats, studied by the isotopic equilibrium method. In Paoletti, R. and Kritchevsky, D., eds. *Advances in lipid res.* Vol. 5. Pp. 209-237 Academic Press, New York.
- Christophe, J. and Mayer, J. 1958. Effect of exercise on glucose uptake in rats and men. *J. Appl. Physiol.* 13:269.
- Connel, A. M., Cooper, J. and Redfearn, J. W. 1958. The contrasting effects of emotional tension and physical exercise on the excretion of 17-ketogenic steroids and 17-ketosteroids. *Acta Endocrinol.* 27:179.
- Connor, W. E. 1968. Dietary sterols: Their relationship to atherosclerosis. *J. Amer. Dietetic Ass.* 52:202.



- Connor, W. E., Hodges, R. E. and Bleiler, R. 1961a. The serum lipids in men receiving high cholesterol and cholesterol-free diets. *J. Clin. Invest.* 40:894.
- Connor, W. E., Hodges, R. E. and Bleiler, R. E. 1961b. Effect of dietary cholesterol upon serum lipids in man. *J. Lab. Clin. Med.* 57:331.
- Cook, R. P., ed. 1958. Cholesterol: Chemistry, biochemistry, and pathology. Academic Press, New York, N.Y.
- Cooper, R. W., Ritota, M. C., Steinberg, M. and Vincelette, R. B. 1966. Hearts and lipids of veteran handball players, a preliminary report. *Angiology* 17:192.
- Cornforth, J. W. 1955. Biosynthesis of cholesterol. *Revs. Pure and Appl. Chem. (Australia)* 4:275.
- Cornforth, J. W., Youhotsky-Gore I. and Popják, G. 1956. The origin of the carbon atoms of cholesterol. *Biochem. J.* 64:38p.
- Cornforth, J. W., Youhotsky-Gore, I. and Popják, G. 1957. Studies on the biosynthesis of cholesterol: 4. Degradation of rings C and D. *Biochem. J.* 65:94.
- Cox, G. E., Taylor, C. B., Cox, L. G. and Counts, M. A. 1958. Atherosclerosis in Rhesus monkeys. I. Hypercholesteremia induced by dietary fat and cholesterol. *Arch. Pathol.* 66:32.
- Cuthbertson, W.F.J., Elcoate, P. V., Ireland, D. M., Mills, D.C.B. and Shearley, P. 1960. Effect of compounds related to thyroxine on serum and liver cholesterol and on atherosclerosis and heart weights in rats and mice. *J. Endocrinol.* 21:45.
- Cutting, W. C., Rytan, D. A. and Tainter, M. L. 1934. Relationship between blood cholesterol and increased metabolism from dinitrophenol and thyroid. *J. Clin. Invest.* 13:547.
- Dam, H. 1958. Historical introduction. In Cook, R. P., ed. Cholesterol: Chemistry, biochemistry, and pathology. Pp. 1-14. Academic Press, New York, N.Y.
- Danielsson, H., Einarsson, K. and Johansson, G. 1967. Effect of biliary drainage on individual reactions in the conversion of cholesterol to taurocholic acid. *Europe. J. Biochem.* 2:44.

- Dauber, D. V. and Katz, L. N. 1942. Experimental cholesterol atheromatosis in an omnivorous animal, the chick. Arch. Pathol. 34:937.
- Dauber, D., Horlick, L. and Katz, L. N. 1949. The role of desiccated thyroid and potassium iodide in cholesterol-induced atherosclerosis of chicken. Amer. Heart J. 38:25.
- Davis, O. F., Beck, C., Bergal, M., Sloan, N. and Levine, A. J. 1962. Some observations of the relationships between atherosclerosis, hyperlipemia, and D-thyroxine. Amer. Heart J. 64:7.
- Dawson, P. M. 1935. The physiology of physical education. Williams and Wilkins Co., Baltimore, Md.
- Dayton, S., Dayton, J., Drimmer, F. and Kendall, F. E. 1960. Rates of acetate turnover and lipid synthesis rates in normal, hypothyroid, and hyperthyroid rats. Amer. J. Physiol. 199:71.
- Debons, A. F. and Schwartz, I. L. 1961. Dependence of the lipolytic action of epinephrine in vitro upon thyroid hormone. J. Lipid Res. 2:86.
- Deming, Q. B., Mosbach, E. H., Bevans, M., Daly, M. M., Abel, L. L., Martin, E., Brun, L. M., Halpern, E. and Kaplan, R. 1958. Blood pressure, cholesterol content of serum and tissues, and atherogenesis in the rat. J. Exp. Med. 107:581.
- Dietschy, J. M. and Siperstein, M. D. 1967. Effect of cholesterol feeding and fasting on sterol synthesis in seventeen tissues of the rat. J. Lipid Res. 8:97.
- Dietschy, J. M. and Wilson, J. D. 1968. Cholesterol synthesis in the squirrel monkey: relative rates of synthesis in various tissues and mechanisms of control. J. Clin. Invest. 47:166.
- Donaldson, H. H. 1932. On the effects of exercise carried through seven generations on the weight of the musculature and on the composition and weight of several organs of the albino rat. Amer. J. Anat. 50:359.
- Donaldson, H. H. 1933. On the effect of exercise beginning at different ages on the weight of the musculature and of several organs of the albino rat. Amer. J. Anat. 53:403.

- Donaldson, H. H. 1935. Effect of prolonged rest following exercise on organ weights of albino rat. Amer. J. Anat. 56:45.
- Duncan, C. H. and Best, M. M. 1958a. Effect of thiouracil on serum and level cholesterol of the athyreotic rat. Amer. J. Physiol. 194:351.
- Duncan, C. H. and Best, M. M. 1958b. Thyroxine-like compounds and cholesterol metabolism: differences in the effects of thyroxine, triiodothyronine, and their formic acid analogues. Endocrinology 63:169.
- Ellefson, R. D. and Mason, H. L. 1962. Effects of thyroid hormone on lipid metabolism in the rat. Endocrinology 71:425.
- Entenman, C., Chaikoff, I. L. and Reichert, F. L. 1942. Blood lipids of the hypophysectomized - thyroidectomized dog. Endocrinology 30:802.
- Epstein, A. A. and Lande, H. 1922. Studies on blood lipoids. I. The relation of cholesterol and protein deficiency to basal metabolism. Arch. Intern. Med. 30:563.
- Eriksson, S. 1956. Influence of thyroid hormone on the formation of bile acids. Acta Chem. Scand. 10:1055.
- Eriksson, S. 1957a. Biliary excretion of bile acids and cholesterol in bile fistula rats. Bile acids and steroids. Proc. Soc. Exp. Biol. Med. 94:578.
- Eriksson, S. 1957b. Influence of thyroid activity on excretion of bile acids and cholesterol in the rat. Proc. Soc. Exp. Biol. Med. 94:582.
- Escobar del Rey, F. and Morreal de Escobar, G. 1956. Studies on the peripheral disappearance of thyroid hormone. Acta Endocrinol. 23:400.
- Eskelson, C. D., Cazee, C. R., Anthony, W., Towne, J. C. and Walske, B. R. 1970. In vitro inhibition of cholesterol-genesis by various thyroid hormone analogs. J. Med. Chem. 13:215.
- Ewan, R. 1969. Triglyceride. Unpublished paper. Ames, Iowa, Iowa State University of Science and Technology, Department of Animal Science.

- Felt, V. 1966. Thyroid and steroid hormones and their relation to serum lipid concentrations in man. *J. Clin. Endocrinol. Metab.* 26:383.
- Fieser, L. F. and Fieser, M. 1949. Natural products related to phenanthrene. 3rd ed. Reinhold, New York, N.Y.
- Fimognari, G. M. and Rodwell, V. W. 1965. Cholesterol biosynthesis: Mevalonate synthesis inhibited by bile salts. *Science* 147:1038.
- Fishman, J., Hellman, L., Zumoff, B. and Gallagher, T. F. 1962. Influence of thyroid hormone on estrogen metabolism in man. *J. Clin. Endocrinol. Metab.* 22:389.
- Fishman, J., Hellman, L., Zumoff, B. and Gallagher, T. F. 1965. Effect of thyroid on hydroxylation of estrogen in man. *J. Clin. Endocrinol. Metab.* 25:365.
- Fitzgerald, O., Hefferman, A. and McFarlane, R. 1965. Serum lipids and physical activity in normal subjects. *Clin. Sci.* 28:83.
- Fleischmann, W. and Wilkins, L. 1941. Sterol balance in hypothyroidism. *J. Clin. Endocrinol.* 1:799.
- Fleischmann, W. and Shumacher, H. 1942. The relationship between serum cholesterol and total body cholesterol in experimental hyper- and hypothyroidism. *Bull. John Hopkins Hosp.* 71:175.
- Fleischmann, W. and Fried, I. A. 1945. Studies on the mechanism of the hypercholesterolemia and hypercalcemia induced by estrogen in immature chicks. *Endocrinology* 36:406.
- Fleischmann, W., Schumacher, H. B. and Wilkins, L. 1940. The effect of thyroidectomy on serum cholesterol and basal metabolic rate in the rabbit. *Amer. J. Physiol.* 131:317.
- Fletcher, K. and Myant, N. B. 1958. Influence of the thyroid on the synthesis of cholesterol by liver and skin in vitro. *J. Physiol.* 144:361.
- Fletcher, K. and Myant, N. B. 1960. Effects of thyroxine on the synthesis of cholesterol and fatty acids by cell-free fractions of rat liver. *J. Physiol.* 154:145.
- Flock, E. V. and Bollman, J. L. 1963. The effect of thiouracil on the metabolism of L-thyroxine. *Biochem. J.* 84:621.

- Foldes, F. F. and Murphy, A. J. 1946. Distribution of cholesterol, cholesterol esters, and phospholipid phosphorus in blood in thyroid disease. *Proc. Soc. Exp. Biol. Med.* 62:218.
- Forbes, J. C. 1944. Effect of thyroxine on neutral fat and cholesterol content of body and liver of rats. *Endocrinology* 35:126.
- Forbes, W., Steele, M. H. and Munro, H. N. 1964. Adrenal hypertrophy in rabbits fed with cholesterol. *Brit. J. Nut.* 18:55.
- Fox, S. M., III. and Skinner, J. S. 1964. Physical activity and cardiovascular health. *Amer. J. Cardiol.* 14:731.
- Fox, S. M., III. and Haskell, W. L. 1968. Physical activity and the prevention of coronary heart disease. *Bull. N.Y. Acad. Med.* 44:950.
- Frantz, I. D., Jr., Schneider, H. S. and Hinkelman, B. T. 1954. Suppression of hepatic cholesterol synthesis in the rat by cholesterol feeding. *J. Biol. Chem.* 206:465.
- Fredrickson, D. S. 1956. The conversion of cholesterol-4-C<sup>14</sup> to acids and other products by liver mitochondria. *J. Biol. Chem.* 222:109.
- Frenkel, R. and Csabay, L. 1962. Effect of regular muscular activity on adrenocortical function in rats. *J. Sports Med.* 2:207.
- Friedberg, S. J., Sher, P. B., Bogdonoff, M. D. and Estes, E. H. 1963. The dynamics of plasma free fatty acid metabolism during exercise. *J. Lipid Res.* 4:34.
- Friedman, M. and Byers, S. O. 1955. Observations concerning the production and excretion of cholesterol in mammals. XVI. The relationship of the liver to the content and control of plasma cholesterol ester. *J. Clin. Invest.* 34:1369.
- Friedman, M., Byers, S. O. and Michaelis, F. 1951. Production and excretion of cholesterol in mammals. IV. Role of liver in restoration of plasma cholesterol after experimentally induced hypocholesteremia. *Amer. J. Physiol.* 164:789.

- Friedman, M., Byers, S. O. and St. George, S. 1956. Cholesterol metabolism. *Ann. Rev. Biochem.* 25:613.
- Friedman, M., Byers, S. O. and Elek, S. R. 1970. Pituitary growth hormone essential for regulation of serum cholesterol. *Nature* 225:464.
- Fukushima, D. K. and Gallagher, T. F. 1952. Isotopic distribution in cholesterol after platinum-catalyzed-hydrogen-deuterium exchange. *J. Biol. Chem.* 198:861.
- Fulmer, H. S. and Roberts, R. W. 1964. Coronary heart disease among the Navajo Indians. *Ann. Intern. Med.* 59:740.
- Furman, R. H. 1969. Endocrine factors in atherogenesis. In Schettler, F. G. and Boyd, G. S., eds. *Atherosclerosis: Pathology, physiology, aetiology, diagnosis and clinical management.* Pp. 410-419. Elsevier Pub. Co., New York, N.Y.
- Gans, J. H. 1960. Bile secretion during experimental hyperthyroidism in the dog. *Amer. J. Physiol.* 199:893.
- Gardner, J. A. and Gainsborough, H. 1928. The relation of plasma cholesterol to basal metabolism. *Brit. Med. J.* ii:935.
- Gertler, M. M., Gorn, S. M. and Sprague, H. B. 1950. Cholesterol, cholesterol esters and phospholipids in health and in coronary artery disease morphology and serum lipids in men. *Circulation* 2:380.
- General Biochemicals. 1968. Catalog Number 11060. General Biochemicals, Chagrin Falls, Ohio.
- Gildea, E. F., Man, E. B. and Peters, J. P. 1938. Serum lipids in hypothyroidism. *J. Clin. Invest.* 18:739.
- Gilligan, D. R., Volk, C. M., Davis, D. and Blumgart, H. L. 1934. Therapeutic effect of total ablation of normal thyroid on congestive heart failure and angina pectoris. VIII. Relationship between cholesterol values, basal metabolic rate and clinical aspects of hypothyroidism. *Arch. Intern. Med.* 54:746.
- Glock, G. E. and McLean, P. 1955. Preliminary investigation of hormonal control of hexose monophosphate oxidative pathway. *Biochem. J.* 61:398.
- Gofman, J. W. and Jones, H. B. 1952. Obesity, fat metabolism and cardiovascular disease. *Circulation* 5:514.

- Gofman, J. W., Tamplin, A. and Strisower, B. 1954. Relation of fat and calorie intake to atherosclerosis. J. Amer. Dietetic Ass. 30:317.
- Goldbloom, A. and Gottlieb, R. 1927. The cholesterol content of the blood of infants and children. Canad. Med. Ass. 17:1333.
- Golding, L. A. 1961. Effects of physical training upon total serum cholesterol levels. Res. Quart. 32(4):499.
- Gollnick, P. D. 1963. Chronic effect of exercise on liver cholesterol of normal and hypercholesteremic rats. Amer. J. Physiol. 205(3):453.
- Gollnick, P. D. and Simmons, S. W. 1967. Physical activity and liver cholesterol. Int. Z. angew. Physiol. einsch. Arbeitsphysiol. 23:332.
- Gordon, H., Lewis, B., Eales, L. and Brock, J. F. 1957. Dietary fat and cholesterol metabolism--fecal elimination of bile acids and other lipids. Lancet ii:1299.
- Gould, R. G. 1951. Lipid metabolism and atherosclerosis. Amer. J. Med. 11:209.
- Gould, R. G. 1958. Biosynthesis of cholesterol. In Cook, R. P., ed. Cholesterol: Chemistry, biochemistry, and pathology. Pp. 209-235. Academic Press, Inc., New York, N.Y.
- Gould, R. G. 1959. The relationship between thyroid hormones and cholesterol biosynthesis and turnover. In Pincus, G., ed. Hormones and atherosclerosis. Pp. 75-88. Academic Press, Inc., New York, N.Y.
- Gould, R. G. and Taylor, C. B. 1950. Effect of dietary cholesterol in hepatic cholesterol synthesis. Fed. Proc. 9:179.
- Gould, R. G. and Popják, G. 1957. Biosynthesis of cholesterol in vivo and in vitro from D,L- $\beta$ -hydroxy- $\beta$ -methyl- $\delta$ -(2- $^{14}$ C)-valerolactone. Biochem. J. 66:51.
- Gould, G. R. and Cook, R. P. 1958. The metabolism of cholesterol and other sterols in the animal organism. In Cook, R. P., ed. Cholesterol: Chemistry, biochemistry, and pathology. Pp. 237-307. Academic Press, Inc., New York, N.Y.

- Gould, R. G. and Swyryd, E. A. 1966. Sites of control of hepatic cholesterol biosynthesis. *J. Lipid Res.* 7:698.
- Gould, R. G., Taylor, C. B., Hegerman, J. S., Warner, I. and Campbell, D. J. 1953. Cholesterol metabolism: I. Effect of dietary cholesterol on the synthesis of cholesterol in dog tissue in vitro. *J. Biol. Chem.* 201:519.
- Grant, L. F. and Schube, P. G. 1934. Effect of alpha dinitrophenol 1-2-4 on man. *J. Lab. Clin. Med.* 20:56.
- Greene, R., Pearce, J. F. and Rideout, D. F. 1961. Effect of D-thyroxine on serum cholesterol. *Brit. Med. J.* 5239:1572.
- Gries, F. A., Matschinsky, F. and Wieland, O. 1962. Induktion der  $\beta$ -hydroxy- $\beta$ -methylglutaryl-reductase durch schilddrusen hormone. *Biochim. Biophys. Acta* 56:615.
- Grossie, J. and Turner, C. W. 1961. Effect of hyperthyroidism on body weight and feed consumption in male and female rats. *Proc. Soc. Exp. Biol. Med.* 107:520.
- Grossie, J. and Turner, C. W. 1965. Effect of thyro-parathyroidectomy and adrenalectomy on food intake in rats. *Proc. Soc. Exp. Biol. Med.* 118:25.
- Grossie, J., Hendrich, C. E. and Turner, C. W. 1964. Comparative methods for determining biological half-life ( $t^{1/2}$ ) of L-thyroxine in normal thyroidectomized and methimazole treated female rats. *Proc. Soc. Exp. Biol. Med.* 120:413.
- Gruder, W., Nolte, I. and Wieland, O. 1968. The influence of thyroid hormones on  $\beta$ -hydroxy- $\beta$ -methylglutaryl-coenzyme A reductase of rat liver. *Europe. J. Biochem.* 4:273.
- Gsell, D. and Mayer, J. 1962. Low blood cholesterol associated with high-caloric, high-saturated-fat intakes in a Swiss Alpine Village population. *Amer. J. Clin. Nut.* 10:471.
- Hahn, D. W., Ishibashi, T. and Turner, C. W. 1965. Effect of hypophysectomy on feed intake in rats. *Proc. Soc. Exp. Biol. Med.* 119:1238.



- Hamburger, J. I., Smith, R. W., Mellinger, R. C. and Miller, J. M. 1961. Epinephrine mobilization of FFA's in Grave's disease and hypothyroidism. Clin. Res. 9:180.
- Handler, P. 1948. The influence of thyroid activity on the liver and plasma lipids of choline- and cysteine-deficient rats. J. Biol. Chem. 173:295.
- Hanson, D. L., Lorenzen, J. A., Morris, A. E., Ahrens, R. A. and Wilson, J. E., Jr. 1967. Effects of fat intake and exercise on serum cholesterol and body composition of rats. Amer. J. Physiol. 213:347.
- Hardin, D. H. 1965. The use of the laboratory rat in exercise experimentation. Res. Quart. 36:370.
- Harris, G. W. 1955. The reciprocal relationship between the thyroid and adrenocortical response to stress. In Wolstenholme, G.E.W. and Cameron, M. P., eds. The human adrenal cortex. Pp. 531-550. Little, Brown and Co., Boston, Mass.
- Haslewood, G.A.D. 1955. Recent developments in our knowledge of bile acids. Physiol. Rev. 35:178.
- Havel, R. J. and Goldfien, A. 1961. The role of the liver and of extrahepatic tissues in the transport and metabolism of fatty acids and triglycerides in the dog. J. Lipid Res. 2:389.
- Havel, R. J., Felts, J. M. and Duyne, C. M. Van. 1962. Formation and fate of endogenous triglycerides in blood plasma of rabbits. J. Lipid Res. 3:297.
- Havel, R. J., Pernow, B. and Jones, N. L. 1967. Uptake and release of free fatty acids and other metabolites in the legs of exercising men. J. Appl. Physiol. 23:90.
- Hearn, G. R. and Wainio, W. W. 1956. Succinic dehydrogenase activity of the heart and skeletal muscle of exercised rats. Amer. J. Physiol. 185:348.
- Hebbelinck, M. and Casier, H. 1966. Effect of muscular exercise on the metabolism of 4-C<sup>14</sup> labelled cholesterol in mice. Int. Z. angew. Physiol. einsch. Arbeitsphysiol. 22:185.

- Hegsted, D. M., Andrus, S. B., Gotsis, A. and Portman, O. W. 1957. The quantitative effects of cholesterol, cholic acid, and type of fat on serum cholesterol and vascular sudanophilia in the rat. *J. Nut.* 63:273.
- Hein, F. V. and Ryan, A. J. 1960. The contributions of physical activity to physical health. *Res. Quart.* 31:263.
- Hellman, L., Bradlow, H. L., Zumoff, B. and Gallagher, T. F. 1961. The influence of thyroid hormone on hydrocortisone production and metabolism. *J. Clin. Endocrinol. Metab.* 21:1231.
- Heyden, S. 1969. Epidemiology: 5. Physical activity versus inactivity. In Schettler, F. G. and Boyd, G. S., eds. *Atherosclerosis: Pathology, physiology, aetiology, diagnosis and clinical management.* Pp. 245-269. Elsevier Pub. Co., New York, N.Y.
- Hill, S. R., Goetz, F. C., Fox, H. M., Muravski, B. J., Krakauer, L. J., Reifenstem, R. W., Gray, S. J., Reddy, W. J., Hedberg, S. E., Mary, J. R., St. and Thron, G. W. 1956. Studies on adrenocortical and psychological response to stress in man. *Arch. Intern. Med.* 97:269.
- Hoffman, A. A., Nelson, W. R. and Goss, F. A. 1967. Effects of an exercise program on plasma lipids of senior Air Force officers. *Amer. J. Cardiol.* 20:516.
- Holloszy, J. O. 1963. The epidemiology of coronary heart disease: National differences and the role of physical activity. *J. Amer. Geriatr. Soc.* 11:718.
- Holloszy, J. O., Skinner, J. S., Toro, G. and Cureton, T. K. 1964. Effects of a six month program of endurance exercise on the serum lipids of middle-aged men. *Amer. J. Cardiol.* 14:753.
- Horlick, L. and Havel, R. 1948. The effect of feeding propylthiouracil and cholesterol on blood cholesterol and arterial intima in the rat. *J. Lab. Clin. Med.* 33:1029.
- Horning, M. G., Fredrickson, D. S. and Anfinsen, C. B. 1957. Studies on the enzymatic degradation of the cholesterol side chain. II. Requirements of the mitochondrial system. *Arch. Biochem. Biophys.* 71:266.

- Horst, A., Rozyrkowa, D. and Zagorska, I. 1960. The influence of emotion on cholesterol metabolism in the white rat. *Acta Med. Pol.* 1:11.
- Hotta, S. and Chaikoff, I. L. 1955. The role of the liver in the turnover of plasma cholesterol. *Arch. Biochem.* 56:28.
- Huff, J. W., Filfillan, J. L. and Hunt, V. M. 1963. Effect of cholestyramine, a bile acid binding polymer, on plasma cholesterol and fecal bile acid excretion in the rat. *Proc. Soc. Exp. Biol. Med.* 114:352.
- Hunter, J. and Wong, L. 1961. Plasma cholesterol levels in New Zealand. *Brit. Med. J.* 5250:486.
- Hurxthal, L. M. 1933a. Blood cholesterol in thyroid disease. I. Analyses of findings in toxic and nontoxic goiter before treatment. *Arch. Intern. Med.* 51:22.
- Hurxthal, L. M. 1933b. Blood cholesterol in thyroid disease. II. Effect of treatment. *Arch. Intern. Med.* 52:86.
- Hurxthal, L. M. 1934a. Blood cholesterol and thyroid disease. III. Myxedema and hypercholesterolemia. *Arch. Intern. Med.* 53:762.
- Hurxthal, L. M. 1934b. Blood cholesterol and hypometabolism. *Arch. Intern. Med.* 53:825.
- Hutchens, T. T., Van Bruggen, J. T., Cockburn, R. M. and West, E. S. 1954. The effect of fasting upon tissue lipogenesis in the intact rat. *J. Biol. Chem.* 208:115.
- Irvine, C.H.G. 1967. Thyroxine secretion rate in the horse in various physiological states. *J. Endocrinol.* 39:313.
- Ivy, A. C., Karvinen, E., Lin, T. M. and Ivy, E. K. 1957. Some parameters of sterol metabolism in man on a sterol and fat-free diet. *J. Appl. Physiol.* 11:1.
- Jansen, G. R., Zanetti, M. E., and Hutchison, C. F. 1966. Studies on lipogenesis *in vivo*: Effects of starvation and re-feeding, and studies on cholesterol synthesis. *Biochem. J.* 99:333.
- Jepson, E. M. 1963. Long-term trial of D-thyroxine in hypercholesterolaemia. *Brit. Med. J.* 5343:1446.

- Johnson, M. L. 1956. Relative importance of inactivity and overeating in the energy balance of obese high school girls. *Amer. J. Clin. Nut.* 4:37.
- Johnson, T. F. and Wong, H.Y.C. 1961. Effect of exercise on plasma cholesterol and phospholipids in college swimmers. *Res. Quart.* 32:514.
- Jolliffe, N. 1959. Fats, cholesterol, and coronary heart disease: A review of recent progress. *Circulation* 20:109.
- Jones, E. M., Montoye, H. J., Johnson, P. B., Martin Martin, J. J., Sister, Huss, W. D. Van. and Cederquist, D. 1964. Effects of exercise and food restriction on serum cholesterol and liver lipids. *Amer. J. Physiol.* 207(2):460.
- Jones, N. L. and Havel, R. J. 1967. Metabolism of free fatty acids and cholesteryl triglycerides during exercise in rats. *Amer. J. Physiol.* 213:824.
- Kahn, H. A. 1963. The relationship of reported coronary heart disease mortality to physical activity of work. *Amer. J. Public Health* 53:1058.
- Kannel, W. B. 1966. The Framingham heart study, habits and coronary disease. U.S. Dept. of H.E.W. Public Health Service Publ. No. 1515.
- Kannel, W. B. and McNamara, P. M. 1967. Physical activity and risk of fatal coronary heart disease. The Framingham study. *Circulation, Suppl. II*, 35-36:154 (abstr.).
- Karp, A. and Stetten, D., Jr. 1949. The effect of thyroid activity on certain anabolic processes studied with the aid of deuterium. *J. Biol. Chem.* 179:819.
- Karvonen, M. J., Pekkarinen, M., Maetsälä, P. and Rautanen, Y. 1961. Diet and serum cholesterol of lumberjacks. *Brit. J. Nut.* 15:157.
- Karvonen, M. J., Orma, E., Punsar, S., Kallio, V., Arstila, M., Luomanmaki, K. and Takkinen, J. 1970. VI. Five-year experience in Finland. In Keys, A., ed. *Coronary heart disease in seven countries*. P. I-52. Am. Heart Ass. Monograph No. 29.

- Katz, L. N. and Stamler, J. 1953. Experimental Atherosclerosis. Thomas Pub. Co., Springfield, Ill.
- Kay, W. W. and Whitehead, R. 1935. The suprarenal in cholesterol-fed rabbits. J. Pathol. Bact. 41:293.
- Kendall, E. C. 1939. The influence of some of the ductless glands in metabolic processes. Endocrinology 24:798.
- Kessler, B. and Leaderer, H. 1966. Fluorometric measurement of triglycerides. In Technicon Symposium. Automation in Analytical Chemistry. Medial, Inc., New York.
- Keys, A. 1952. The cholesterol problem. Voeding 13:539.
- Keys, A., Mickelsen, O., Miller, E. O. and Chapman, C. B. 1950. The relations in man between cholesterol levels in the diet and in the blood. Science 112:79.
- Keys, A., Anderson, J. T., Aresu, M., Biörck, G., Brock, J. F., Bronte-Stewart, B., Fidanza, F., Keys, M. H., Malmros, H., Poppi, A., Postele, T., Swahn, B. and del Vecchio, A. 1956a. Physical activity and the diet in populations differing in serum cholesterol. J. Clin. Invest. 35: 1173.
- Keys, A., Anderson, J. T. and Mickelsen, O. 1956b. Serum cholesterol in men in basal and nonbasal states. Science 123:29.
- Keys, A., Anderson, J. T. and Grande, F. 1957. Serum cholesterol response to dietary fat. Lancet i:787.
- Kimeldorf, D. J. and Baum, S. J. 1954. Alterations in organ and body growth of rats following daily exhaustive exercise, X-irradiation, and post-irradiation exercise. Growth 18:79.
- Kleiber, M. 1961. The fire of life: An introduction to animal energetics. John Wiley and Sons, Inc., New York, N.Y.
- Kobernick, S. D. and Hashimoto, T. 1963. Histochemistry of atherosclerosis: II. Spontaneous degenerative lesions of aorta of exercised and sedentary rabbits. Lab. Invest. 12:685.
- Kobernick, S. D. and Niwayama, G. 1960. Physical activity in experimental cholesterol atherosclerosis in rabbits. Amer. J. Pathol. 36:393.

- Kobernick, S. D., Niwayama, G. and Zuchlewski, A. C. 1957. Effect of physical activity in experimental cholesterol atherosclerosis in rabbits. *Proc. Soc. Exp. Biol. Med.* 96:623.
- Kon, G.A.R. and Woolman, A. M. 1939. Saprogin. Part III. The dehydrogenation products of methylsarsasaprogenin and methylcholestanol. *J. Chem. Soc.* 1939:794.
- Kratzing, C. C. 1965. Influence of partial immersion and exercise in lipid metabolism in the rat. *Amer. J. Physiol.* 209:78.
- Kritchevsky, D. 1958. Cholesterol. John Wiley and Sons, Inc., New York, N.Y.
- Kritchevsky, D. 1960. Influence of thyroid hormones and related compounds on cholesterol biosynthesis and degradation: A review. *Metabolism* 9:984.
- Kritchevsky, D. 1967. Influence of thyroactive compounds on lipid metabolism and experimental atherosclerosis. Estratto da *Il Giornale Dell'Atherosclerosi* 5(2):175.
- Kritchevsky, D. and Tepper, S. A. 1965. Oxidation of cholesterol by rat liver mitochondria: Effect of thyroidectomy. *J. Cell. Comp. Physiol.* 66:91.
- Kritchevsky, D. and Tepper, S. A. 1967. Influence of D- and L-thyroxine on pre-established atheromata in rabbits: Effects of mode of administration. *J. Lipid Res.* 7:103.
- Kritchevsky, D., Staple, E. and Whitehouse, M. W. 1960. Regulation of cholesterol biosynthesis and catabolism. *Amer. J. Clin. Nut.* 8:411.
- Kritchevsky, D., Moynihan, J. L. and Sachs, M. L. 1961. Influence of thyroactive compounds on serum and liver cholesterol in rats. *Proc. Soc. Exp. Biol. Med.* 108:254.
- Kritchevsky, D., Cottrell, M. C. and Tepper, S. A. 1962. Oxidation of cholesterol by rat liver mitochondria: Effect of thyroactive compounds. *J. Cell. Comp. Physiol.* 60:105.
- Kumaresan, P. and Turner, C. W. 1967. Effect of advancing age on thyroid hormone secretion rate of male and female rats. *Proc. Soc. Exp. Biol. Med.* 124:752.

- Kunde, M. M., Green, M. F. and Burns, G. 1932. Blood changes in experimental hypo- and hyperthyroidism (rabbit). Amer. J. Physiol. 99:469.
- Kurland, G. S., Lucas, J. L. and Freedberg, A. S. 1961. The metabolism of intravenously infused C<sup>14</sup>-labeled cholesterol in euthyroidism and myxedema. J. Lab. Clin. Med. 57:574.
- Lang, C. M. 1967. Effects of psychic stress on atherosclerosis in the squirrel monkey. Proc. Soc. Exp. Biol. Med. 126:30.
- Langdon, R. G. and Bloch, K. 1953. The effect of some dietary additions on the synthesis of cholesterol from acetate in vitro. J. Biol. Chem. 202:77.
- Lashof, J. C., Bondy, P. K., Sterling, K. and Man, E. B. 1954. Effect of muscular exercise on circulating thyroid hormone. Proc. Soc. Exp. Biol. Med. 86:233.
- Lawry, E. Y., Mann, G. V., Peterson, A., Wysocki, A. P., O'Connell, R. and Stare, F. J. 1957. Cholesterol and  $\beta$ -lipoproteins in the serum of Americans. Amer. J. Med. 22:605.
- Lee, K. T., Kim, D. N., Han, Y. S. and Goodale, F. 1962. Geographic studies of atherosclerosis. The effect of a strict vegetarian diet on serum lipid and electrocardiographic patterns. Arch. Environ. Health 4:4.
- Lee, V. A. 1967. Individual trends in the total serum cholesterol of children and adolescents over a ten-year period. Amer. J. Clin. Nut. 20:5.
- Lepp, A., Wagle, S. R. and Oliner, L. 1964. Effects of L- and D-thyroxine on cholesterol synthesis and turnover in the chick. Proc. Soc. Exp. Biol. Med. 115:517.
- Lewis, L. A., Olmsted, F., Page, I. H., Lawry, E. Y., Man, G. V., Stare, F. J., Hanig, M., Lauffer, M. A., Gordon, T. and Moore, F. E. 1957. Serum lipid levels in normal persons: Findings of a co-operative study of lipoproteins and atherosclerosis. Circulation 16:227.
- Lewis, L. A., Page, H. I. and Brown, H. B. 1961. Effects of exercise on serum and hepatic lipids in rats fed high fat diets. Amer. J. Physiol. 201:4.

- Lin, T. H., Rubinstein, R. and Holmes, W. L. 1963. A study of the effect of D- and L-triiodothyronine on bile acid excretion of rats. *J. Lipid Res.* 4:63.
- Lindsey, C. D. and Wilson, J. D. 1965. Evidence for a contribution by the intestinal wall to the serum cholesterol of the rat. *J. Lipid Res.* 6:173.
- Lindsted, S. and Samuelsson, B. 1959. Bile acids and sterioids: LXXXIII. On the interconversion of cholic and deoxycholic acid in the rat. *J. Biol. Chem.* 234:2026.
- Linn, T. C. 1967. The effect of cholesterol feeding and fasting upon  $\beta$ -hydroxy- $\beta$ -methylglutaryl coenzyme A reductase. *J. Biol. Chem.* 242:990.
- Lipsky, S. R., Bondy, P. K., Man, E. B. and McGuire, J. S. 1955. Effects of triiodothyronine on biosynthesis of plasma lipids from acetate-1-C<sup>14</sup> in myxedematous subjects. *J. Clin. Invest.* 34:950.
- Lynen, F. 1955. Lipid metabolism. *Ann. Rev. Biochem.* 24:653.
- Lyon, T. P., Yankley, A., Gofman, J. W. and Strisower, B. 1956. Lipoproteins and diet in coronary heart disease. *Calif. Med.* 84:325.
- Lyons, B. E. and Cashman, C. W. 1941. The effects of hypophysectomy upon hypercholesterolemia of dogs. *Endocrinology* 28:715.
- McArdle, W. D. and Montoye, H. J. 1966. Reliability of exhaustive swimming in the laboratory rat. *J. Appl. Physiol.* 21:1431.
- McArdle, W. D. and Montoye, H. J. 1967. Relationship of organ weight and swimming performance in the albino rat. *Res. Quart.* 38:671.
- McClimtock, J. T., Hines, H. M. and Jordan, D. P. 1939. Effects of activity on tissues of rat. *Proc. Soc. Exp. Biol. Med.* 42:139.
- McDonough, J. R., Hames, C. G., Stulb, S. C. and Garrison, G. E. 1965. Coronary heart disease among negroes and whites in Evans County, Georgia. *J. Chronic. Dis.* 18:443.



- McGee, L. C. 1935. Blood cholesterol in disturbances of the metabolic rate. *Ann. Intern. Med.* 9:728.
- McMillan, G. C., Klatzo, I. and Duff, G. L. 1954. Histochemical and staining reactions of tissues and organs of cholesterol-fed rabbits. *Lab. Invest.* 3:451.
- Malhotra, S. L. 1967. Serum lipids, dietary factors, and ischemic heart disease. *Amer. J. Clin. Nut.* 20:462.
- Malinow, M. R. and Perley, A. 1969. The effects of physical exercise on cholesterol degradation in man. *J. Atheroscler. Res.* 10:107.
- Malinow, M. R., Gimeno, A. L., Pellegrino-Iraldi, A. A., Fernandez-Gimeno, M. A., Bur, G. E., Lacuara, J. L. and Moguilevsky, J. A. 1960. The orientation of the C-17-OH group of oestradiol in relation to its antiatherogenic activity. *J. Endocrinol.* 21:1.
- Malinow, M. R., McLaughlin, P. and Perley, A. 1968a. Cholesterol: Treadmill activity accelerates oxidation in rats. *Science* 160:1239.
- Malinow, M. R., McLaughlin, P. and Perley, A. 1968b. The effect of muscular concentration on cholesterol oxidation. *J. Appl. Physiol.* 25:733.
- Malinow, M. R., Perley, A. and McLaughlin, P. 1969. Muscular exercise and cholesterol degradation: Mechanisms involved. *J. Appl. Physiol.* 27:662.
- Malinow, M. R., McLaughlin, P., Perley, A., Laasteun, L. and Hook, E. Van. 1970. Hepatic and adrenal degradation of cholesterol during rest and muscular activity. *J. Appl. Physiol.* 29:323.
- Malmros, H. and Wigand, G. 1957. The effect on serum-cholesterol on diets containing different fats. *Lancet* ii:1.
- Mann, G. V., Andrus, S. B., McNally, A. M. and Stare, F. J. 1953. Experimental atherosclerosis in Cebus monkeys. *J. Exp. Med.* 98:195.
- Mann, G. V., Munoz, J. A., and Scrimshaw, N. S. 1955a. The serum lipoproteins and cholesterol concentrations of Central and North Americans with different dietary habits. *Amer. J. Med.* 19:25.

- Mann, G. V., Nicol, B. M. and Stare, F. J. 1955b. The beta-lipoprotein and cholesterol in sera of Nigerians. Brit. Med. J. 1946:1008.
- Mann, G. V., Teel, K., Olive, H., McNally, A. and Bruno, D. 1955c. Exercise in the disposition of dietary calories. New Engl. J. of Med. 253:349.
- Martínez, B. D. 1917. Travaux du lab de physiologie de la faculte de med. de Buenos Aires, 1914. Endocrinology 1:357.
- Marx, W., Marx, L. and Shimoda, F. 1950. Thyroid hormone and tissue cholesterol distribution. Proc. Soc. Exp. Biol. Med. 73:599.
- Marx, W., Gustin, S. T. and Leve, C. 1953. Effects of thyroxine, thyroidectomy and lowered environmental temperature on incorporation of deuterium into cholesterol. Proc. Soc. Exp. Biol. Med. 83:143.
- Mason, R. L., Hunt, H. M. and Hurxthal, L. 1930. Blood cholesterol values in hyperthyroidism and hypothyroidism -- their significance. New Engl. J. Med. 203:1273.
- Masoro, E. J. 1968. Physiological chemistry of lipids in mammals. W. B. Saunders Co., Philadelphia, Penn.
- Mathur, K. S. 1960. Environmental factors in coronary heart disease. An epidemiological study at Agra (India). Circulation 21:684.
- Mayer, J. 1968. Overweight--causes, cost, and control. Prentice Hall, Inc., Englewood Cliffs, N.J.
- Mayer, J. and Thomas, D. W. 1967. Regulation of food intake and obesity. Science 156:328.
- Mayer, J., Marshall, N. B., Vitale, J. J., Christensen, J. H., Mashayekhi, M. B. and Stare, F. J. 1954. Exercise, food intake and body weight in normal rats and genetically obese adult mice. Amer. J. Physiol. 177:544.
- Melichar, F. 1965. Plasma cholesterol and phospholipids in various occupational groups. J. Atheroscler. Res. 5:432.

- Mendelsohn, D., Mendelsohn, L. and Staple, E. 1966. The in vitro catabolism of cholesterol: Formation of cholic acid from cholesterol in rat liver. *Biochemistry* 5:3194.
- Merskey, C., Gordon, H. and Lackner, H. 1960. Blood coagulation and fibrinolysis in relation to coronary heart disease. *Brit. Med. J.* 5168:219.
- Miettinen, T. A. 1968. Mechanism of serum cholesterol reduction by thyroid hormones in hypothyroidism. *J. Lab. Clin. Med.* 71:537.
- Migicovsky, B. B. and Wood, I. D. 1955. Effect of starvation on cholesterol biosynthesis in vitro. *Canad. J. Biochem.* 33:858.
- Miller, D. S. and Payne, P. R. Weight maintenance and food intake. 1962. *J. Nut.* 78:255.
- Mitropoulos, K. A. and Myant, N. B. 1964. Effect of thyroid hormone upon the metabolism of cholesterol by isolated liver mitochondria. *Biochem. J.* 91:20p.
- Montoye, H. J., Huss, W. D. Van, Brewer, W. D., Jones, E. M., Ohlson, M. A., Mahoney, E. and Olson, H. 1959. Effects of exercise on blood cholesterol in middle-aged men. *Amer. J. Clin. Nut.* 7:139.
- Montoye, H. J., Sherburne, J., Ackerman, K., Jones, E. M. and Cederquist, D. 1962. Effects of exercise and milk consumption on blood serum cholesterol in rats. *Res. Quart.* 33(3):430.
- Moore, R. B., Anderson, J. T., Taylor, H. L., Keys, A. and Frantz, I. D. 1968. Effect of dietary fat on the fecal excretion of cholesterol and its degradation products in man. *J. Clin. Invest.* 47:1517.
- Morreale de Escobar, G. and Escobar del Rey, F. 1967. Extrathyroid effects of some antithyroid drugs and their metabolic consequences. *Recent Progress in Hormone Res.* 23:87.
- Morris, J. and Crawford, M. 1958. Coronary heart disease and physical activity of work. *Brit. Med. J.* 2:1485.
- Moses, C. 1952. Dietary cholesterol and atherosclerosis. *Amer. J. Med. Sci.* 224:212.

- Moutafis, C. D. and Myant, N. B. 1968. Increased hepatic synthesis of cholesterol after ileal by-pass in monkeys. Clin. Sci. 34:541.
- Myant, N. B. 1964. The thyroid and lipid metabolism. In Paoletti, R., ed. Lipid Pharmacology. Pp. 299-323. Academic Press, Inc., New York, N.Y.
- Myant, N. B. and Eder, H. A. 1961. The effect of biliary drainage upon the synthesis of cholesterol in the liver. J. Lipid Res. 2:363.
- Myasnikov, A. L. 1958. Influence of some factors on development of experimental cholesterol atherosclerosis. Circulation 17:99.
- Naito, H. K. and Griffith, D. R. 1971a. Automated apparatus for manual tissue-grinders. Amer. Biol. Teach. 33:108.
- Naito, H. K. and Griffith, D. R. 1971b. Rapid and simplified method for total hepatic cholesterol analysis. Submitted for publication. J. Lipid Res.
- Nikkilä, E. A., Torsti, P. and Penttilä, O. 1963. The effect of exercise on lipoprotein lipase activity of rat heart, adipose tissue and skeletal muscle. Metabolism 12:863.
- Naughton, J. and Balke, B. 1964. Physical working capacity in medical personnel and the response of serum cholesterol to acute exercise and to training. Amer. J. Med. Sci. 247:286.
- Nejad, N. S. and Chaikoff, I. L. 1963. L-thyroxine on acetate conversion to cholesterol by livers of hypophysectomized rats. Amer. J. Physiol. 204:137.
- O'Hara, D. D., Porte, D. and Williams, R. H. 1966. The effect of diet and thyroxine on plasma lipids in myxedema. Metabolism 15:123.
- Okay, R. 1959. Dietary fat and cholesterol metabolism: Effects of unsaturation of dietary fats on liver and serum lipids. Metabolism 8:241.
- Olivecrona, T. and Sjövall, J. 1959. Bile acids in rat portal blood. Acta Physiol. Scand., Suppl. 46:284.

- Oliver, M. F. and Boyd, G. S. 1959. Thyroid and estrogen treatment of hypercholesterolemia. In Pincus, G., ed. Hormones and atherosclerosis. Pp. 403-419. Academic Press, Inc., New York, N.Y.
- Oppenheimer, J. H., Surks, M. I. and Schwartz, H. L. 1969. The metabolic significance of exchangeable cellular thyroxine. In Astwood, E. B., ed. Recent progress in hormone research. Vol. 25. Pp. 381-422. Academic Press, Inc., New York, N.Y.
- Orma, E. J. 1957. Effect of physical activity on atherosclerosis: An experimental study in cockerels. Acta Physiol. Scand. 41:1.
- Page, I. H. and Brown, H. B. 1952. Induced hypercholesterolemia and atherogenesis. Circulation 6:681.
- Page, I. H. and Menschick, W. 1932. The destruction of cholesterol by the animal organism. J. Biol. Chem. 97:359.
- Papacostas, C. A., Sevy, R. W., Ohler, E. A., Bello, C. T. and Brenes, L. 1960. Plasma 17-hydroxycorticosteroid levels in normotensive and hypertensive subjects and the influence of posture. Amer. J. Med. Sci. 239:745.
- Papadopoulos, N. M., Bloor, C. M. and Strandefor, J. C. 1969. Effects of exercise and training on plasma lipids and lipoproteins in the rat. J. Appl. Physiol. 26:760.
- Patek, P. R., Bernick, S., Ershoff, B. H. and Wells, A. 1963. Induction of atherosclerosis by cholesterol feeding in the hypophysectomized rat. Amer. J. Pathol. 42:137.
- Pedley, F. G. 1942. Coronary disease and occupation. Canad. Med. Ass. J. 46:147.
- Peters, J. P. and Man, E. B. 1943. The interrelationship of serum lipid in patients with thyroid disease. J. Clin. Invest. 22:715.
- Peters, J. P. and Man, E. B. 1950. The significance of serum cholesterol in thyroid disease. J. Clin. Invest. 29:1.
- Pitt-Rivers, R. and Tata, J. R. 1959. The thyroid hormones. Vol. 1. Pergamon Press, New York, N.Y.
- Pomeroy, W. and White, P. D. 1958. Coronary heart disease in former football players. J. Amer. Med. Ass. 167:711.

- Popják, G. 1958. Biosynthesis of cholesterol and related substances. *Ann. Rev. Biochem.* 27:533.
- Porte, D., O'Hara, D. D. and Williams, R. H. 1966. The relation between postheparin lipolytic activity and plasma triglyceride in myxedema. *Metabolism* 15:107.
- Pothier, L. and Italic, T. Van. 1960. Role of the thyroid in lipid mobilization. *Clin. Res.* 8:377.
- Pruett, E. D. 1970. FFA mobilization during and after prolonged severe muscular work in men. *J. Appl. Physiol.* 29:809.
- Raab, W. and Krzywanek, H. J. 1965. Cardiovascular sympathetic tone and stress response related to personality patterns and exercise habits. *Amer. J. Cardiol.* 16:42.
- Renold, A. E., Quingley, T. B., Kennard, H. E. and Thorn, G. W. 1951. Reaction of the adrenal cortex to physical and emotional stress in college oarsman. *New Engl. J. Med.* 244:754.
- Rhodes, B. 1967. Effect of exercise on the thyroid gland. *Nature* 216:917.
- Ring, G. C., Bosch, M. and Lo, C. S. 1970. Effects of exercise on growth, resting metabolism, and body composition of Fischer rats. *Proc. Soc. Exp. Biol. Med.* 133:1162.
- Rochelle, R. H. 1961. Blood plasma cholesterol changes during a physical training program. *Res. Quart.* 32:538.
- Romanowski, W. and Strazynski, W. 1968. Effect of exercise on the tissue respiration of the brain skeletal muscle, heart, liver, kidneys, and spleen. *Int. Z. angew. Physiol. einsch. Arbeitsphysiol.* 25:329.
- Rosenman, R. H., Byers, S. O. and Friedman, M. 1952a. The mechanism responsible for the altered blood cholesterol content in deranged thyroid states. *J. Clin. Endocrinol. Metab.* 12:1287.
- Rosenman, R. H., Friedman, M. and Byers, S. O. 1952b. Observations concerning the metabolism of cholesterol in the hypo- and hyperthyroid rat. *Circulation* 5:589.

- Saba, N. and Hechter, O. 1955. Cholesterol-4-C<sup>14</sup> metabolism in adrenal homogenates. Fed. Proc. 14:775.
- Sauer, F. 1960. Fatty acid, cholesterol, and acetoacetate biosynthesis in liver homogenates from normal and starved guinea pigs. Canad. J. Biochem. 38:635.
- Scaife, J. F. and Migicovsky, B. B. 1957. Cholesterol biosynthesis: The starvation block. Canad. J. Biochem. 35:615.
- Schettler, G. 1949. Studien zum cholesterinstoffwechsel der maus. Biochem. Z. 319:444.
- Schilling, F. J., Christakis, G. J., Bennett, N. J. and Coyle, J. F. 1964. Studies of serum cholesterol in 4,244 men and women: An epidemiological and pathogenetic interpretation. Amer. J. Public Health 54:461.
- Schmidt, L. H. and Huges, H. B. 1938. Free and total cholesterol content of whole blood and plasma as related to experimental variations in thyroid activity. Endocrinology 22:475.
- Schönheimer, R. and Breusch, F. 1933. Synthesis and destruction of cholesterol in the organism. J. Biol. Chem. 103:439.
- Scrimshaw, N. S. 1964. Nutrition and stress. In Wolstenholme, G.E.W. and O'Connor, M., eds. Diet and body constitution. Pp. 40-58. Little, Brown, and Co., Boston, Mass.
- Sharper, A. G. 1962. Cardiovascular studies in the Samburu tribe of northern Kenya. Amer. Heart J. 63:437.
- Sharper, A. G. and Jones, K. W. 1959. Serum cholesterol, diet and coronary heart disease in Africans and Asians in Uganda. Lancet ii:534.
- Sharper, A. G., Jones, M. and Kyobe, J. 1961. Plasma-lipids in an African tribe living on a diet of milk and meat. Lancet ii:1324.
- Shipley, R. E., Pfeiffer, R. R., March, M. M. and Anderson, R. C. 1958. Sitosterol feeding: Chronic animal and clinical toxicology and tissue analysis. Cir. Res. 6:373.

- Shoppee, C. W. 1956. Steroids. In Todd, Sir Alexander, ed. Perspectives in organic chemistry. Pp. 315-346. Interscience, New York, N.Y.
- Shoppee, C. W. 1964. Chemistry of the steroids. Butterworths, Washington, D.C.
- Shoppee, C. W. and Shoppee, E. 1953. Sex hormones; adrenocortical hormones. In Rodd, E. H., ed. Chemistry of carbon compounds. Vol. 2. Part B. Pp. 765-875. Elsevier Publishing Co., Amsterdam, Netherlands.
- Simko, V. and Babala, J. 1964. Physical activity and the fat metabolism in rats. Med. Exp. 10:286.
- Simko, V., Nemec, R. and Ginter, E. 1970. Incorporation of acetate- $C^{14}$  into liver cholesterol of rats subjected to prolonged exercise. Experimentia 26:749.
- Simpson, E. R. and Boyd, G. S. 1968. Studies on the conversion of cholesterol to pregnenolone in bovine adrenal mitochondria. In McKerns, K. W., ed. Functions of the adrenal cortex. Vol. I. Pp. 49-76. Appleton-Century-Crofts, New York, N.Y.
- Siperstein, M. D. and Chaikoff, I. L. 1952.  $C^{14}$ -Cholesterol: III. Excretion of carbons 4 and 26 in feces, urine, and bile. J. Biol. Chem. 198:93.
- Siperstein, M. D. and Chaikoff, I. L. 1955. Formation of bile acids. Fed. Proc. 14:767.
- Siperstein, M. D. and Murray, A. W. 1957. Cholesterol metabolism in man. J. Clin. Invest. 34:1449.
- Siperstein, M. D. and Guest, M. J. 1960. Studies on the site of the feedback control of cholesterol synthesis. J. Clin. Invest. 39:642.
- Siperstein, M. D. and Fagan, V. M. 1966. Feedback control of mevalonate synthesis by dietary control. J. Biol. Chem. 241:602.
- Siperstein, M. D., Jayko, M. E., Chaikoff, I. L. and Dauben, W. G. 1952. Nature of the metabolic products of  $C^{14}$ -cholesterol excreted in bile and feces. Proc. Soc. Exp. Biol. Med. 81:720.
- Snedecor, G. W. and Cochran, W. G. 1967. Statistical methods. 6th edition. Iowa State University Press, Ames, Iowa.



- Spain, D. M. and Bradess, V. A. 1960. Occupational physical activity and the degree of coronary atherosclerosis in normal men. *Circulation* 22:239.
- Sperry, W. M. and Webb, M. 1950. The effect of increasing age on serum cholesterol concentration. *J. Biol. Chem.* 187:107.
- Stamler, J. 1958. Diet and atherosclerotic disease: III. Epidemiologic findings. *J. Amer. Dietetic Ass.* 34:929.
- Stamler, J., Silber, E. N., Miller, A. J., Akman, L., Bolene, C. and Katz, L. N. 1950. The effect of thyroid- and of dinitrophenol-induced hypermetabolism of plasma and tissue lipids and atherosclerosis in the cholesterol-fed chick. *J. Lab. Clin. Med.* 35:351.
- Stamler, J., Lindberg, H. A., Berkson, D. M., Shaffer, A., Miller, W. and Poindexter, A. 1960. Prevalence and incidence of coronary heart disease in strata of the labor force of a Chicago-industrial corporation. *J. Chron. Dis.* 11:405.
- Stamler, J., Berkson, M., Lindberg, H. A., Miller, W., Hall, Y. and Stamler, R. 1962. Relationship of weight to hypercholesterolemia, hypertension and electrocardiographic abnormalities in middle-aged Chicagoans. *Circulation* 26:672.
- Staple, E. and Rabinowitz, J. L. 1962. Formation of trihydroxycoprostanic acid from cholesterol in man. *Biochim. Biophys. Acta* 59:735.
- Stefanik, P. A., Heald, F. P., Jr. and Mayer, J. 1959. Caloric intake in relation to energy output of obese and non-obese adolescent boys. *Amer. J. Clin. Nut.* 7:55.
- Steiner, A. and Kendall, F. E. 1946. Atherosclerosis and arteriosclerosis in dogs following ingestion of cholesterol and thiouracil. *Arch. Pathol.* 42:433.
- Stevenson, J.A.F. 1967. Exercise, food intake and health in experimental animals. *Canad. Med. Ass. J.* 96:862.
- Stevenson, J.A.F., Box, B. M., Feleki, V. and Beaton, J. R. 1966. Bouts of exercise and food intake in the rat. *J. Appl. Physiol.* 21:118.

- Story, J. A. and Griffith, D. R. 1971. Total cholesterol content of Wayne Lab-Blox. Unpublished report. Iowa State University of Science and Technology, Department of Zoology and Entomology. Ames, Iowa.
- Strand, O. 1962. Influence of propylthiouracil and D- and L-triiodothyronine on excretion of bile acids in bile Fistula rats. Bile acids and steroids 118. Proc. Soc. Exp. Biol. Med. 109:668.
- Strand, O. 1963. Effects of D- and L-triiodothyronine and of propylthiouracil in the production of bile acids in the rat. J. Lipid Res. 4:305.
- Stuart, K. L., Schneckloth, R. E., Lewis, L. A., Moore, F. E. and Córcoran, A. C. 1962. Diet, serum cholesterol, protein, blood haemoglobin and glycosuria in a West Indian community (St. Kitts, W.I.) with observations on ischaemic heart disease. Brit. Med. J. 5315:1283.
- Stulb, S. C., McDonough, J. R., Greenberg, B. G. and Hames, C. G. 1965. The relationship of nutrient intake and exercise to serum cholesterol levels in white males in Evans County, Georgia. Amer. J. Clin. Nut. 16:238.
- Suld, H. M., Staple, E. and Gurin, S. 1962. Mechanism of formation of bile acids from cholesterol: Oxidation of 5 $\beta$ -cholestane-3 $\alpha$ , 7 $\alpha$ , 12 $\alpha$ -triol and formation of propionic acid from the side chain by rat liver mitochondria. J. Biol. Chem. 237:338.
- Suzuki, T. 1966. Effect of physical exercise on adrenal 17-hydroxycorticosteroid secretion rate in the dog. In Raab, W., ed. Prevention of ischemic heart disease. P. 504. Charles C. Thomas, Springfield, Ill.
- Tabachnick, I. I. and Bonnycastle, D. D. 1954. Effect of thyroxine on coenzyme A content of some tissues. J. Biol. Chem. 207:757.
- Tanabe, Y., Komiyama, T., Kuboto, D. and Tamaki, Y. 1965. Comparison of the effects of thiouracil, propylthiouracil, and methimazole on I<sup>131</sup> metabolism by the chick thyroid and measurements of thyroxine secretion rates. Gen. Comp. Endocrinol. 5:60.
- Tapley, D. G., Davidoff, F., Hatfield, W. B. and Ross, J. E. 1959. Physiological disposition of D- and L-thyroxine in the rat. Amer. J. Physiol. 197:1021.

- Taylor, C. B. and Gould, R. G. 1950. Effect of dietary cholesterol on rate of cholesterol synthesis in the intact animal measured by means of radioactive carbon. *Circulation* 2:467.
- Taylor, C. B., Nelson, L., Stumpe, L., Cox, G. and Tamura, R. 1956. Hepatic cholesterol synthesis: Its depression by dietary cholesterol and subsequent recovery on a cholesterol-free diet. *Fed. Proc.* 15:543.
- Taylor, H. L., Anderson, J. T. and Keys, A. 1957. Physical activity, serum cholesterol and other lipids in man. *Proc. Soc. Exp. Biol. Med.* 95:383.
- Taylor, H. L., Anderson, J. T. and Keys, A. 1961. Studies on diet, physical activity, and serum cholesterol concentration. *Circulation* 24:1055 (abstr.).
- Taylor, H. L., Blackburn, H., Brozek, J., Parlin, R. W. and Puchner, T. 1967. Railroad employees in the United States. In Keys, A., ed. *Epidemiological studies related to coronary heart disease, characteristics of men aged 40-59 in seven countries.* *Acta Med. Scand. Suppl.* 460.
- Technicon Instruments Corporation. 1965. AutoAnalyzer method file, cholesterol N-24a. Total cholesterol in serum. Technicon Instruments Corp., Ardsley, N.Y.
- Thomas, B. M. and Miller, A. T., Jr. 1958. Adaptation to forced exercise in the rat. *Amer. J. Physiol.* 193:350.
- Thomas, C. B. and Garn, S. M. 1960. Degree of obesity and serum cholesterol level. *Science* 131:42.
- Thomas, W. A. and Hartroft, W. S. 1959. Myocardial infarction in rats fed diets containing high fat, cholesterol, thiouracil, and sodium cholate. *Circulation* 19:65.
- Thompson, J. C. and Vars, H. M. 1953a. Daily cholic acid and cholesterol excretion in the bile of hypo-, hyper-, and euthyroid rats. *Fed. Proc.* 12:404.
- Thompson, J. C. and Vars, H. M. 1953b. Biliary excretion of cholic acid and cholesterol in hyper-, hypo-, and euthyroid rats. *Proc. Soc. Exp. Biol. Med.* 83:246.
- Thompson, J. C. and Vars, H. M. 1954. Influence of thyroid activity on the hepatic excretion of cholic acid and cholesterol. *Amer. J. Physiol.* 179:405.

- Tipton, C. M., Terjung, R. L. and Barnard, R. J. 1968. Response of thyroidectomized rats to training. Amer. J. Physiol. 215:1137.
- Tomkins, G. M. and Chaikoff, I. L. 1952. Cholesterol synthesis by liver. I. Influence of fasting and of diet. J. Biol. Chem. 196:569.
- Tomkins, G. M., Sheppard, H. and Chaikoff, I. L. 1953. Cholesterol synthesis by liver. III. Its regulation by ingested cholesterol. J. Biol. Chem. 201:137.
- Tsung-chin, H. and Shih-chen, W. 1965. Regulation of cholesterol metabolism by thyroid hormone. Scientia Sinica 14:874.
- Turner, K. B. 1933. Studies on the prevention of cholesterol atherosclerosis in rabbits; effects of whole thyroid and of potassiumiodide. J. Exp. Med. 58:115.
- Turner, K. B., Present, C. H. and Bidwell, E. M. 1938. The role of the thyroid in the regulation of the blood cholesterol of rabbits. J. Exp. Med. 67:111.
- Uhley, H. N. and Friedman, M. 1959. Blood lipids, clotting, and coronary atherosclerosis in rats exposed to a particular form of stress. Amer. J. Physiol. 197:396.
- Wade, O. L. and Bishop, S. M. 1962. Cardiac output and regional blood flow. Blackwell Scientific Pub., Oxford.
- Wade, P. A. 1929. Clinical and experimental studies on calcium and cholesterol in relation to the thyroid-parathyroid apparatus. Amer. J. Med. Sci. 177:790.
- Walker, W. J. 1953. Relationship of adiposity to serum cholesterol and lipoprotein levels and their modification by dietary means. Ann. Intern. Med. 39:705.
- Walker, W. J., Lawry, E. Y., Love, D. E., Mann, G. V., Levine, S. A. and Stare, F. J. 1953. Effect of weight reduction and caloric balance on serum lipoprotein and cholesterol levels. Amer. J. Med. 14:654.
- Warnock, N. H., Clarkson, T. B. and Stevenson, R. 1957. Effect of exercise on blood coagulation time and atherosclerosis of cholesterol-fed cockerels. Cir. Res. 5:478.

- Weis, H. J. and Dietschy, J. M. 1969. Failure of bile acids to control hepatic cholesterogenesis: Evidence for endogenous cholesterol feedback. *J. Clin. Invest.* 48:2398.
- Weiss, H. S., Brown, F. D., Grüminger, P. and Fisher, H. 1966. Physical activity and atherosclerosis in the adult chicken. *J. Atherosclero. Res.* 6:407.
- Weiss, S. B. and Marx, W. 1955. The fate of radioactive cholesterol in mice with modified thyroid activities. *J. Biol. Chem.* 213:349.
- Wells, A. F. and Ershoff, B. H. 1962. Effects of cholesterol feeding on plasma and liver cholesterol levels in the hypophysectomized rat. *Proc. Soc. Exp. Biol. Med.* 109:643.
- Westra, J. J. and Kunde, M. M. 1933. Blood cholesterol in experimental hypo- and hyperthyroidism, (rabbit). *Amer. J. Physiol.* 103:1.
- Wilber, C. G. and Hunn, J. B. 1960. Swimming of albino mice. *J. Appl. Physiol.* 15:704.
- Wilcox, E. B., Galloway, L. S. and Taylor, F. 1964. Effect of protein, milk intake, and exercise on athletes. *J. Amer. Dietetic Ass.* 44:95.
- Wilson, J. B. and Reinke, R. T. 1968. Transfer of locally synthesized cholesterol from intestinal wall to intestinal lymph. *J. Lipid Res.* 9:85.
- Wolf, S., McCabe, W. R., Yamamoto, J., Adsett, C. A. and Schottsteadt, W. W. 1962. Changes in serum lipids in relation to emotional stress during rigid control of diet and exercise. *Circulation* 16:379.
- Wong, H.Y.C., Simmons, R. L. and Hawthorne, E. W. 1956. Effects of controlled exercise on experimental atherosclerosis in androgen-treated chicks. *Fed. Proc.* 15:203.
- Wong, H.Y.C., Anderson, M. B., Kim, J. K., Liu, D. J. and Hawthorne, E. W. 1957. Hypocholesterolizing effect of exercise on cholesterol-fed cockerels. *Fed. Proc.* 16:138 (abstr.).

- Wong, H.Y.C., Anderson, M. B. and Kimura, T. 1958.  
Changes in blood cholesterol and atherogenesis in  
roosters by exercise and addrogen on plain mash after  
an "atherogenic diet." 1958. Circulation 18:801.
- Wong, H.Y.C., Liu, B., Shim, R. and Johnson, F. D. 1960.  
The influence of exercise on experimental  
atherosclerosis of hens fed atherogenic diet. Fed.  
Proc. 19:16 (abstr.).
- Young, J. W. 1968. Effects of D- and L-thyroxine on enzymes  
in liver and adipose tissue of rats. Amer. J.  
Physiol. 214:378.
- Zyl, A. Van. 1957. Note on the effects of thyroidectomy and  
thyroid hormone administration on the concentration of  
bile cholesterol and cholic acid. J. Endocrinol.  
16:213.
- Zukel, W. J., Lewis, R. H., Enterline, P. E., Painter, R. C.,  
Ralston, L. S., Fawcett, R. M., Meredith, A. P. and  
Peterson, B. 1959. A short-term community study of  
the epidemiology of coronary heart disease. Amer. J.  
Public Health 49:1630.

## APPENDIX

### Tables

Table 1. Coding and description of the twenty treatment combinations<sup>a</sup>

Thyroid status (A)	Physical activity (B)				Mean <sup>b</sup>
	No exercise	Standing	Moderate exercise	Exhaustive exercise	
	Groups <sup>c</sup>				
Euthyroid control (C)	I <sup>d</sup>	II	III	IV	+ <sup>e</sup>
Athyroid (A-Tdx)	V	VI	VII	VIII	+
Hyperthyroid (Hyper-Tdx)	IX	X	XI	XII	+
Euthyroid (Eu-Tdx)	XIII	XIV	XV	XVI	+
Hypothyroid (Hypo-Tdx)	XVII	XVIII	XIX	XX	+
Mean <sup>f</sup>	++ <sup>g</sup>	++	++	++	

<sup>a</sup>n=12 rats per treatment combination.

<sup>b</sup>Average of the four treatment combination means (averaged means).

<sup>c</sup>Twenty treatment combination means.

<sup>d</sup>Simple effects are obtained by subtracting mean values of one treatment combination from another at each level of physical activity or thyroid status.

<sup>e</sup>Main effect of A are obtained by subtracting the averaged means of one thyroid status from the averaged means of another thyroid status.

<sup>f</sup>Average of the five treatment combination means (averaged means).

<sup>g</sup>Main effects of B are obtained by subtracting the averaged means of one level of physical activity from the averaged means of another level of physical activity.



Table 2. Influence of different levels of physical activity and thyroxine on final body weight (mean  $\pm$  S.E.M.)

Thyroid status <sup>b</sup>	Physical activity <sup>a</sup>				Mean
	No exercise	Standing	Moderate exercise	Exhaustive exercise	
Control	425.2 $\pm$ 11.8 <sup>c</sup>	415.7 $\pm$ 4.0	393.9 $\pm$ 8.4 <sup>s</sup>	366.5 $\pm$ 12.3 <sup>†††</sup>	(400.3 $\pm$ 9.1)
Athyroid	306.5 $\pm$ 21.2	298.4 $\pm$ 13.9	284.0 $\pm$ 15.6	299.6 $\pm$ 14.3	(294.9 $\pm$ 16.3) <sup>†††</sup>
Hyperthyroid	376.7 $\pm$ 10.1	378.6 $\pm$ 11.3	376.4 $\pm$ 10.5	375.5 $\pm$ 9.9	(376.8 $\pm$ 10.5) <sup>†††</sup>
Euthyroid	417.6 $\pm$ 11.2	409.5 $\pm$ 8.9	373.8 $\pm$ 10.0 <sup>†††</sup>	359.3 $\pm$ 9.7 <sup>†††</sup>	(390.0 $\pm$ 9.9)
Hypothyroid	406.1 $\pm$ 10.2	393.3 $\pm$ 10.9	381.1 $\pm$ 11.0	376.1 $\pm$ 10.1 <sup>s</sup>	(389.1 $\pm$ 10.5)
Mean	(386.4 $\pm$ 13.9)	(377.3 $\pm$ 13.7)	(361.8 $\pm$ 12.7) <sup>††</sup>	(355.4 $\pm$ 9.2) <sup>†††</sup>	

<sup>a</sup>lsd (least significant difference,  $\alpha = .05$ ) for B = 14.63 grams.

<sup>b</sup>lsd for A = 16.36 grams.

<sup>c</sup>lsd for AB = 32.65 grams.

<sup>s</sup>.05 < P < .1.

<sup>††</sup>P < .01.

<sup>†††</sup>P < .0005.

Table 3. Influence of different levels of physical activity and thyroxine on daily feed intake (g/100 g BW)

Thyroid status <sup>b</sup>	Physical activity <sup>a</sup>				Mean
	No exercise	Standing	Moderate exercise	Exhaustive exercise	
Control	6.40 $\pm$ .40 <sup>c</sup>	6.50 $\pm$ .38	6.66 $\pm$ .19 <sup>*</sup>	6.59 $\pm$ .18	6.54 $\pm$ .29
Athyroid	6.14 $\pm$ .27	5.91 $\pm$ .19	5.96 $\pm$ .04	5.88 $\pm$ .13 <sup>*</sup>	5.97 $\pm$ .16 <sup>***</sup>
Hyperthyroid	7.68 $\pm$ .14	7.23 $\pm$ .26	7.55 $\pm$ .13	7.31 $\pm$ .01 <sup>††</sup>	7.48 $\pm$ .15 <sup>***</sup>
Euthyroid	6.17 $\pm$ .14	6.46 $\pm$ .11	6.34 $\pm$ .20	6.17 $\pm$ .14	6.29 $\pm$ .15 <sup>**</sup>
Hypothyroid	6.43 $\pm$ .25	6.39 $\pm$ .36	6.45 $\pm$ .10	6.28 $\pm$ .06	6.39 $\pm$ .19 <sup>†</sup>
Mean	6.57 $\pm$ .24	6.45 $\pm$ .26 <sup>*</sup>	6.59 $\pm$ .15	6.45 $\pm$ .11 <sup>*</sup>	

<sup>a</sup>lsd for B = 0.11.

<sup>b</sup>lsd for A = 0.12.

<sup>c</sup>lsd for AB = 0.24.

<sup>\*</sup>P < .05.

<sup>†</sup>P < .02.

<sup>††</sup>P < .01.

<sup>\*\*</sup>P < .001.

<sup>\*\*\*</sup>P < .005.

Table 4. Influence of different levels of physical activity and thyroxine on serum total cholesterol concentration (mg/100 ml)

Thyroid status <sup>b</sup>	Physical activity <sup>a</sup>				Mean
	No exercise	Standing	Moderate exercise	Exhaustive exercise	
Control	57.8±2.1 <sup>c</sup>	58.1±2.0	54.8±1.4	51.6±2.3 <sup>§</sup>	55.6±1.9
Athyroid	59.3±2.3	59.5±2.5	59.6±2.2	61.5±3.5 <sup>††</sup>	60.0±2.6 <sup>†</sup>
Hyperthyroid	56.5±2.4	54.4±2.1	54.1±2.0	54.3±1.5	55.6±2.0
Euthyroid	61.1±2.9	56.6±2.0	54.1±1.7*	60.7±2.9 <sup>††</sup>	58.1±2.4*
Hypothyroid	58.3±2.6	59.2±1.9	59.7±2.0	60.6±1.1 <sup>††</sup>	59.4±1.9 <sup>†</sup>
Mean	58.6±2.5	57.6±2.2	56.5±1.5	57.6±2.3	

<sup>a</sup>lsd for B = 4.13 mg/100 ml serum.

<sup>b</sup>lsd for A = 3.25 mg/100 ml serum.

<sup>c</sup>lsd for AB = 6.51 mg/100 ml serum.

<sup>§</sup>.05 < P < .1.

\*P < .05.

<sup>†</sup>P < .02.

<sup>††</sup>P < .01.

Table 5. Influence of different levels of physical activity and thyroxine on total hepatic cholesterol concentration (mg/100 g BW)

Thyroid status <sup>b</sup>	Physical activity <sup>a</sup>				Mean
	No exercise	Standing	Moderate exercise	Exhaustive exercise	
Controls	0.86±.04 <sup>c</sup>	0.87±.02	0.95±.04	.98±.03	0.92±.03
Athyroid	1.34±.09	1.40±.08 <sup>†††</sup>	1.36±.07 <sup>†††</sup>	1.35±.07 <sup>†††</sup>	1.36±.08 <sup>†††</sup>
Hyperthyroid	0.98±.00	0.96±.04	0.95±.04	1.02±.05	0.98±.03
Euthyroid	0.90±.03	0.97±.04	0.97±.04	1.07±.04*	0.98±.04
Hypothyroid	0.90±.04	1.04±.05 <sup>§</sup>	1.02±.06 <sup>§</sup>	1.13±.05**	1.02±.05**
Mean	0.99±.04	1.05±.05	1.05±.05	1.11±.05 <sup>†††</sup>	

<sup>a</sup>1sd for B = .07 mg/100 g BW.

<sup>b</sup>1sd for A = .07 mg/100 g BW.

<sup>c</sup>1sd for AB = .15 mg/100 g BW.

<sup>§</sup>.05 < P < .1.

\*P < .05.

\*\*P < .001.

†††P < .0005.

Table 6. Nose-tail lengths of the twenty treatment combination groups (mm)

Thyroid status	Physical activity				Mean
	No exercise	Standing	Moderate exercise	Exhaustive exercise	
Euthyroid control	427.5 <sup>a</sup>	427.5	413.7	413.9	420.6
Athyroid	382.4	368.7	365.1	369.7	371.4
Hyperthyroid	403.1	414.8	413.2	418.5	412.4
Euthyroid	414.9	409.2	406.3	408.6	409.7
Hypothyroid	409.3	401.5	406.3	408.6	405.6
Mean	407.4	404.3	400.9	403.2	

<sup>a</sup><sub>n</sub> = 12 rats.

Table 7. Total adrenal weights of the twenty treatment combination groups (mg)<sup>a</sup>

Thyroid status	Physical activity				Mean
	No exercise	Standing	Moderate exercise	Exhaustive exercise	
Euthyroid control	50.5	44.1	44.2	51.9	47.6
Athyroid	36.8	32.6	30.8	35.0	33.8
Hyperthyroid	40.9	47.4	45.9	46.7	45.1
Euthyroid	41.3	44.6	45.0	44.2	43.7
Hypothyroid	37.1	39.5	48.9	42.8	42.0
Mean	41.3	41.6	42.9	44.1	

<sup>a</sup>Wet tissue weight.

Table 8. Influence of different levels of physical activity and thyroxine on serum triglyceride concentration (mg/100 ml  $\pm$  SEM)

Thyroid status <sup>b</sup>	Physical activity <sup>a</sup>				Mean
	No exercised	Standing	Moderate exercise	Exhaustive exercise	
Control	88.9 $\pm$ 5.2 <sup>c</sup>	69.5 $\pm$ 4.2 <sup>††</sup>	59.3 $\pm$ 3.3 <sup>††</sup>	55.7 $\pm$ 3.4**	68.4 $\pm$ 4.0
Athyroid	43.7 $\pm$ 2.9**	36.3 $\pm$ 2.3	50.7 $\pm$ 3.1	33.9 $\pm$ 2.1	41.2 $\pm$ 2.7**
Hyperthyroid	47.5 $\pm$ 2.8**	48.8 $\pm$ 4.7	49.1 $\pm$ 2.9	42.1 $\pm$ 2.6	46.9 $\pm$ 3.3**
Euthyroid	57.8 $\pm$ 3.5**	56.9 $\pm$ 3.5	51.5 $\pm$ 3.2	37.9 $\pm$ 2.3 <sup>§</sup>	51.0 $\pm$ 3.1**
Hypothyroid	62.9 $\pm$ 3.8**	48.5 $\pm$ 3.0	50.3 $\pm$ 3.0	32.9 $\pm$ 2.0 <sup>††</sup>	48.7 $\pm$ 2.9**
Mean	60.2 $\pm$ 3.6	58.0 $\pm$ 3.5	52.2 $\pm$ 3.1*	40.5 $\pm$ 2.5**	

<sup>a</sup>lsd (.05) for B = 6.4 mg/100 ml.

<sup>b</sup>lsd (.05) for A = 7.2 mg/100 ml.

<sup>c</sup>lsd (.05) for AB = 14.3 mg/100 ml.

<sup>§</sup>.05 < P < .1.

\*P < .05.

††P < .01.

\*\*P < .001.

Table 9. Swimming time of rats swum until exhausted (minutes)<sup>a</sup>

Thyroid status	Age of rats (weeks)			
	10	15	20	Mean
Controls	13.6 $\pm$ 4.3 <sup>b</sup>	5.2 $\pm$ 1.5	4.1 $\pm$ 0.9	7.6 $\pm$ 2.2
Athyroid	6.3 $\pm$ 2.1	2.3 $\pm$ 0.4	1.0 $\pm$ 0.3	3.2 $\pm$ 0.9*
Hyperthyroid	11.4 $\pm$ 6.5	4.4 $\pm$ 2.6	3.8 $\pm$ 0.8	6.5 $\pm$ 3.3
Euthyroid	9.0 $\pm$ 5.3	3.1 $\pm$ 1.1	2.6 $\pm$ 0.6	4.9 $\pm$ 2.2*
Hypothyroid	8.2 $\pm$ 4.4	2.8 $\pm$ 1.3	2.0 $\pm$ 0.2	4.3 $\pm$ 1.9*
Mean	12.1 $\pm$ 5.5	4.5 $\pm$ 1.5*	2.7 $\pm$ 0.6*	

<sup>a</sup>Mean  $\pm$  S.D.

<sup>b</sup>n = 12 rats.

\*P < .05.



Table 10. Results of analysis of variance on the effects of thyroxine and physical activity on the final body weight , daily feed intake, serum and hepatic total cholesterol, and serum triglyceride concentration

Variates	Due to	Sum of squares	df	Mean square	F	Significance
Final body weight	A <sup>a</sup>	354004.1	4	88501.0	53.1	P < .0005
	B <sup>b</sup>	36128.6	3	12042.9	7.2	P < .0005
	AB <sup>c</sup>	26728.9	12	2227.4	1.3	P > .75
	error	366519.1	220	1665.0		
Daily feed intake	A	58.5	4	14.6	168.0	P < .0005
	B	0.8	3	0.3	3.1	P < .05
	AB	2.6	12	0.2	2.5	P < .005
	error	19.2	220	0.1		
Serum cholesterol	A	828.2	4	207.1	3.1	P < .025
	B	307.9	3	102.6	1.6	P > .10
	AB	1062.0	12	88.5	1.3	P > .10
	error	14555.7	220	66.2		
Hepatic cholesterol	A	5.8	4	1.5	45.0	P < .0005
	B	0.5	3	0.2	5.4	P < .001
	AB	0.4	12	0.0	0.9	P > .50
	error	7.2	220	0.0		
Serum triglyceride	A	19214.6	4	4803.7	15.0	P < .0005
	B	13976.8	3	4658.9	14.6	P < .0005
	AB	14420.4	12	1201.6	3.8	P < .0005
	error	70268.9	220	319.4		

<sup>a</sup>A = thyroid level.

<sup>b</sup>B = level of physical activity.

<sup>c</sup>AB = interaction of thyroid level and physical activity.

Table 11. Results of analysis of covariance on the effects of thyroxine and physical activity on serum and hepatic total cholesterol and serum triglyceride concentration

Variates	Due to	Sum of squares	df	Mean square	F	Significance
Serum cholesterol	A <sup>a</sup>	62912.4	4	15728.1	8.5	P < .0005
	B <sup>b</sup>	324.8	3	108.3	1.6	P > .05
	AB <sup>c</sup>	1054.8	12	87.9	1.3	P < .05
	Error	14390.6	219	65.7		
Hepatic cholesterol	A	5.7	4	1.4	46.7	P < .0005
	B	0.7	3	0.2	7.7	P < .0005
	AB	0.3	12	0.0	0.8	
	Error	6.6	219	0.0		
Serum triglyceride	A	23126.812	4	5781.7	19.2	P < .0005
	B	15841.9	3	5280.6	17.5	P < .0005
	AB	10941.2	12	911.7	3.0	P < .01
	Error	66070.9	219	301.7		

<sup>a</sup>A = thyroid level.

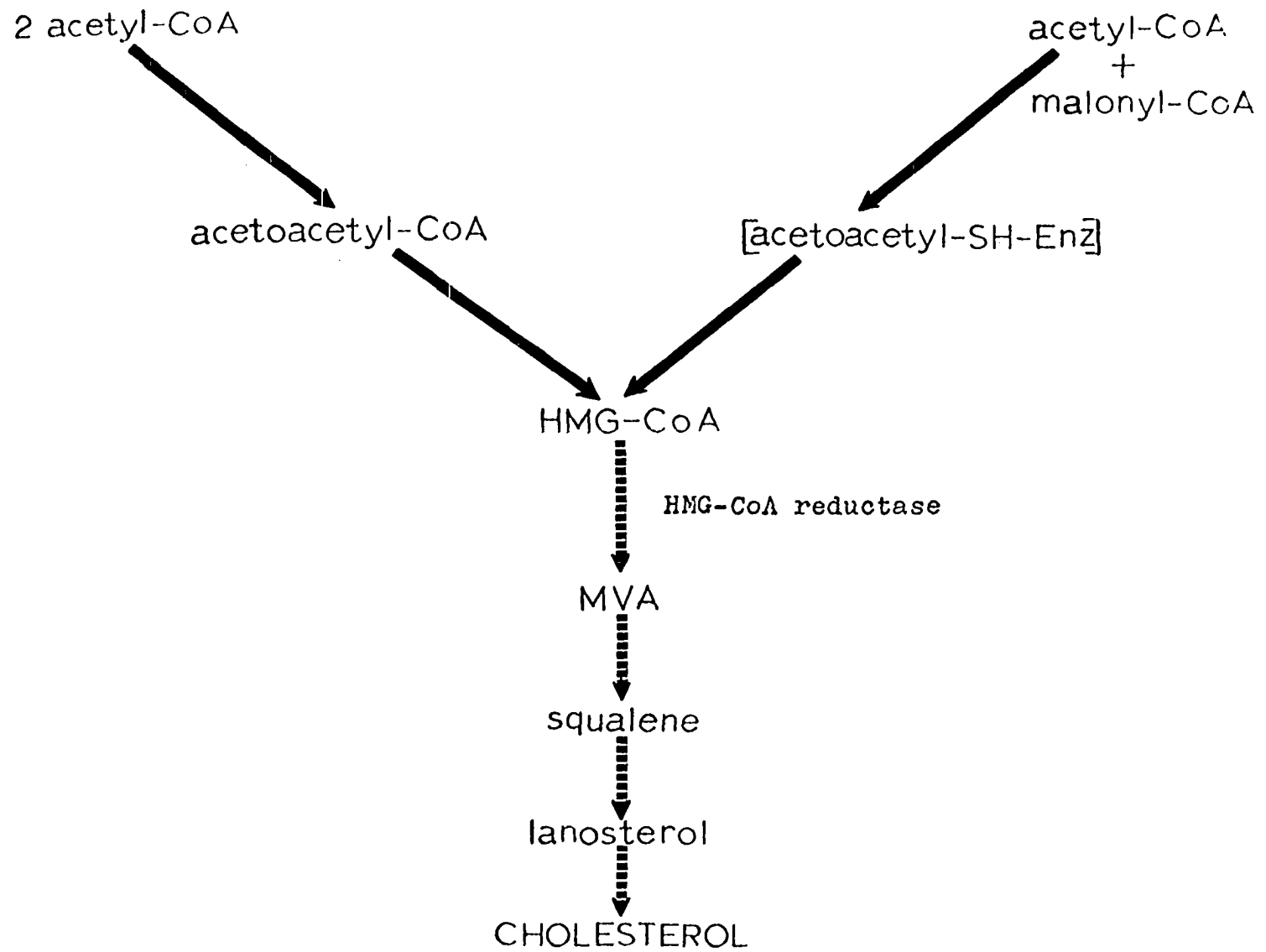
<sup>b</sup>B = level of physical activity.

<sup>c</sup>AB = interaction of thyroid level and physical activity.

Figures

Figure 2. Biochemical pathway for cholesterol synthesis

Only those steps that have demonstrated unequivocal proof for their existence in the metabolic pathway are shown. Figure shows two possible pathways in forming  $\beta$ -hydroxy- $\beta$ -methylglutaryl-coenzyme A (HMG-CoA). Rate-limiting step in cholesterol metabolism is between HMG-CoA and MVA (mevalonic acid).



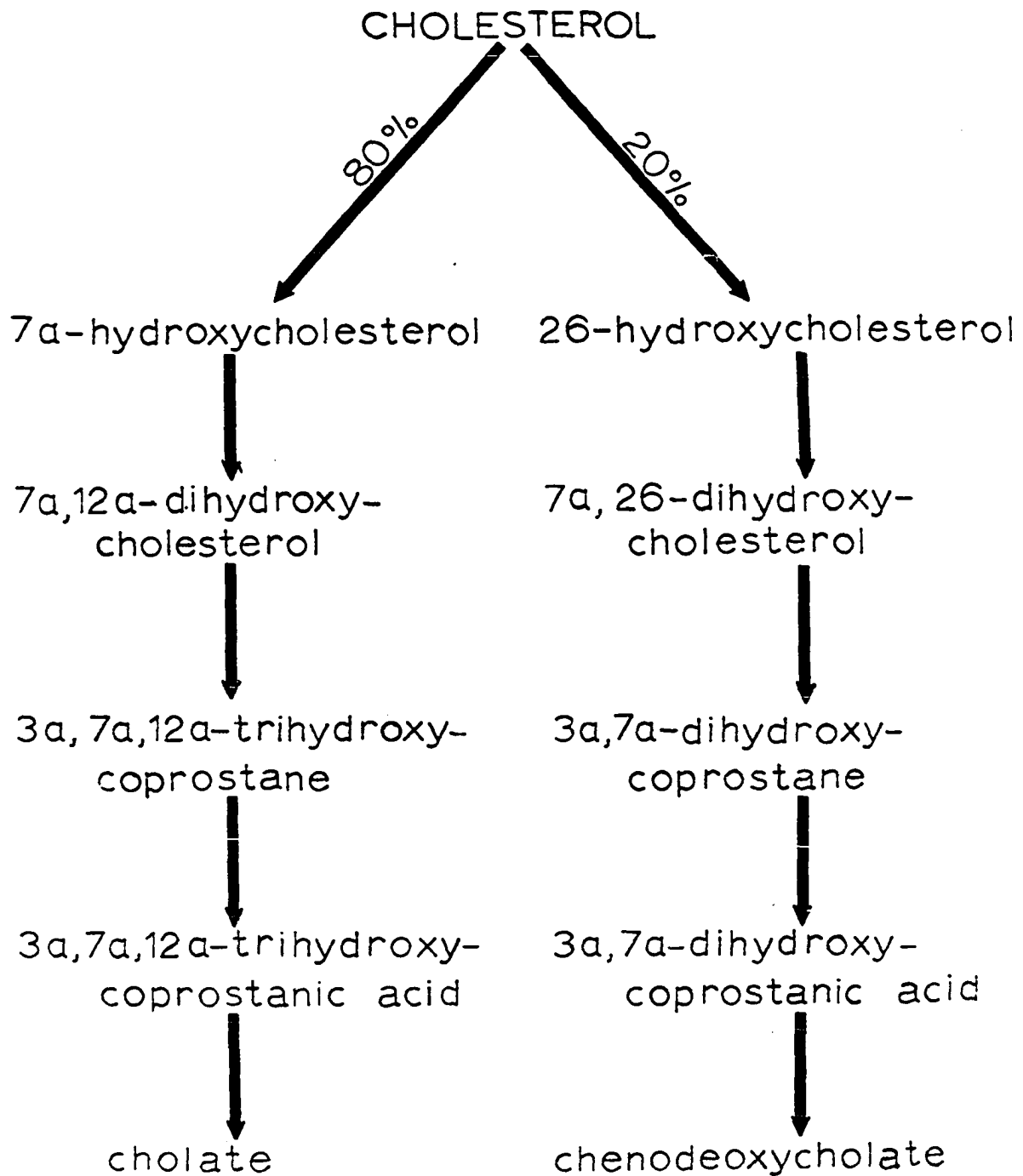


Figure 3. Biochemical pathway for cholesterol catabolism to bile acids in rats

Figure 4. The influence of four levels of physical activity and five levels of thyroid status on the final body weight of rats (grams)

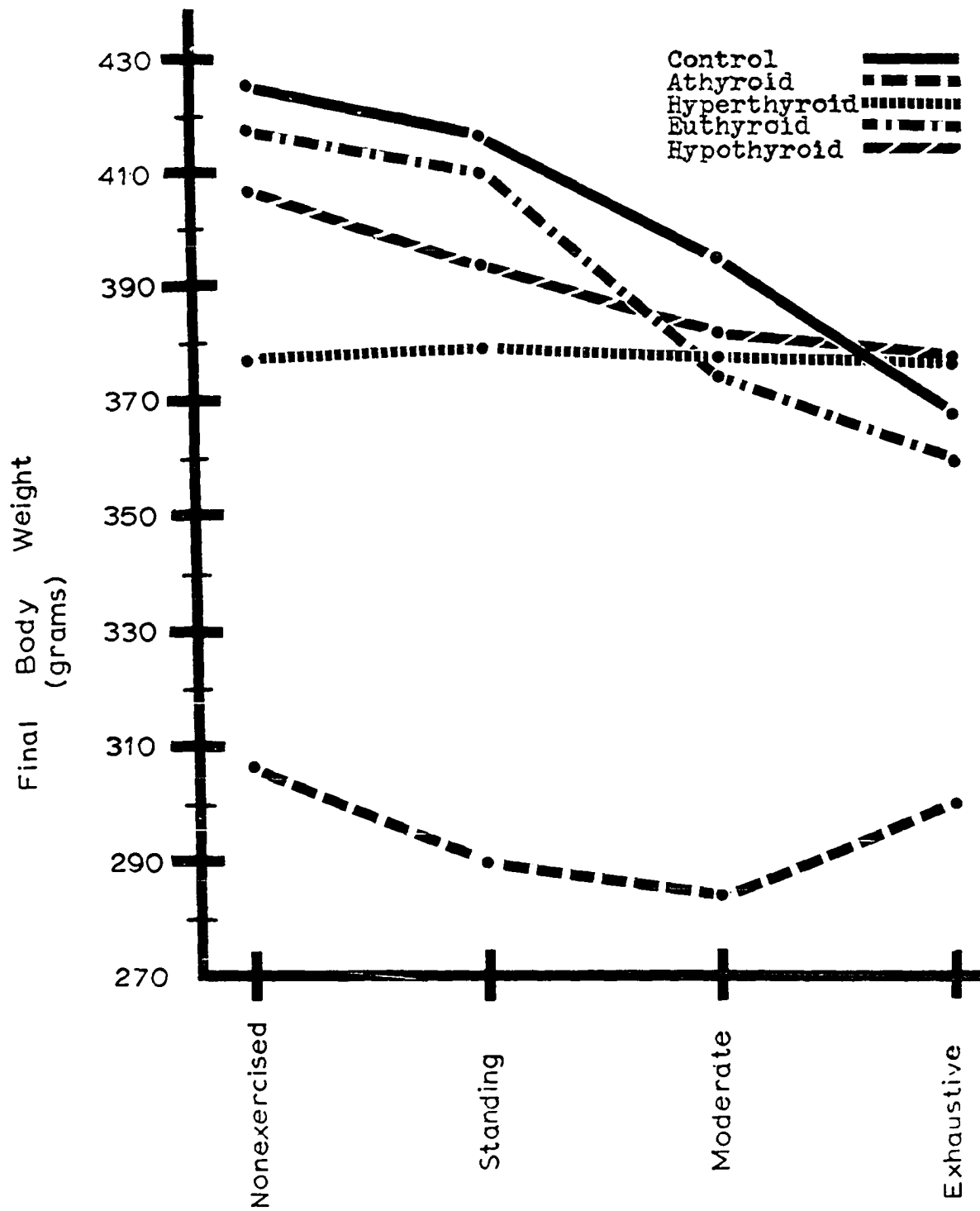




Figure 5. The influence of four levels of physical activity and five levels of thyroid status on daily feed intake (grams per 100 grams body weight)

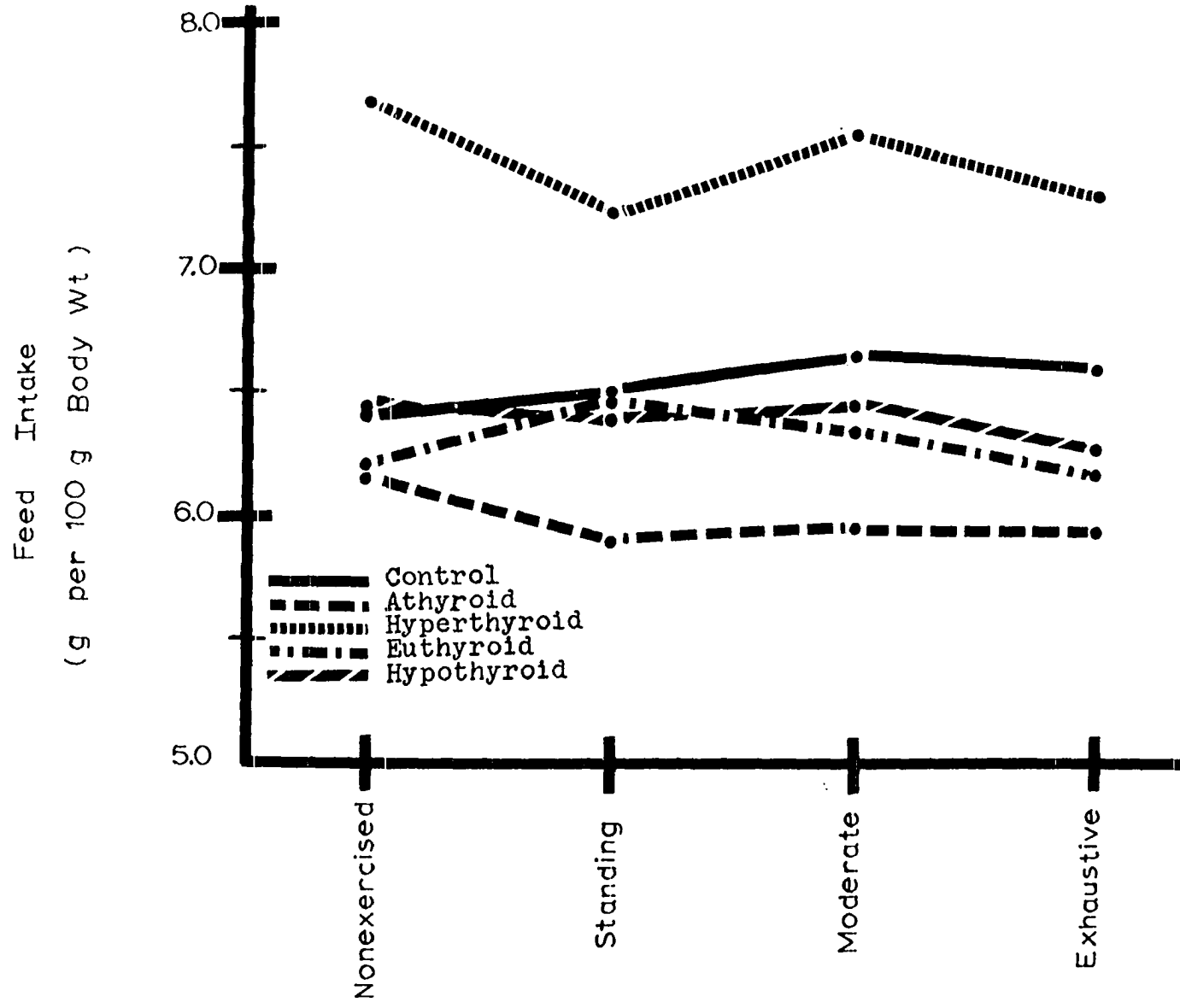


Figure 6. The influence of four levels of physical activity and five levels of thyroid status on serum total cholesterol concentration (milligrams per 100 ml serum)

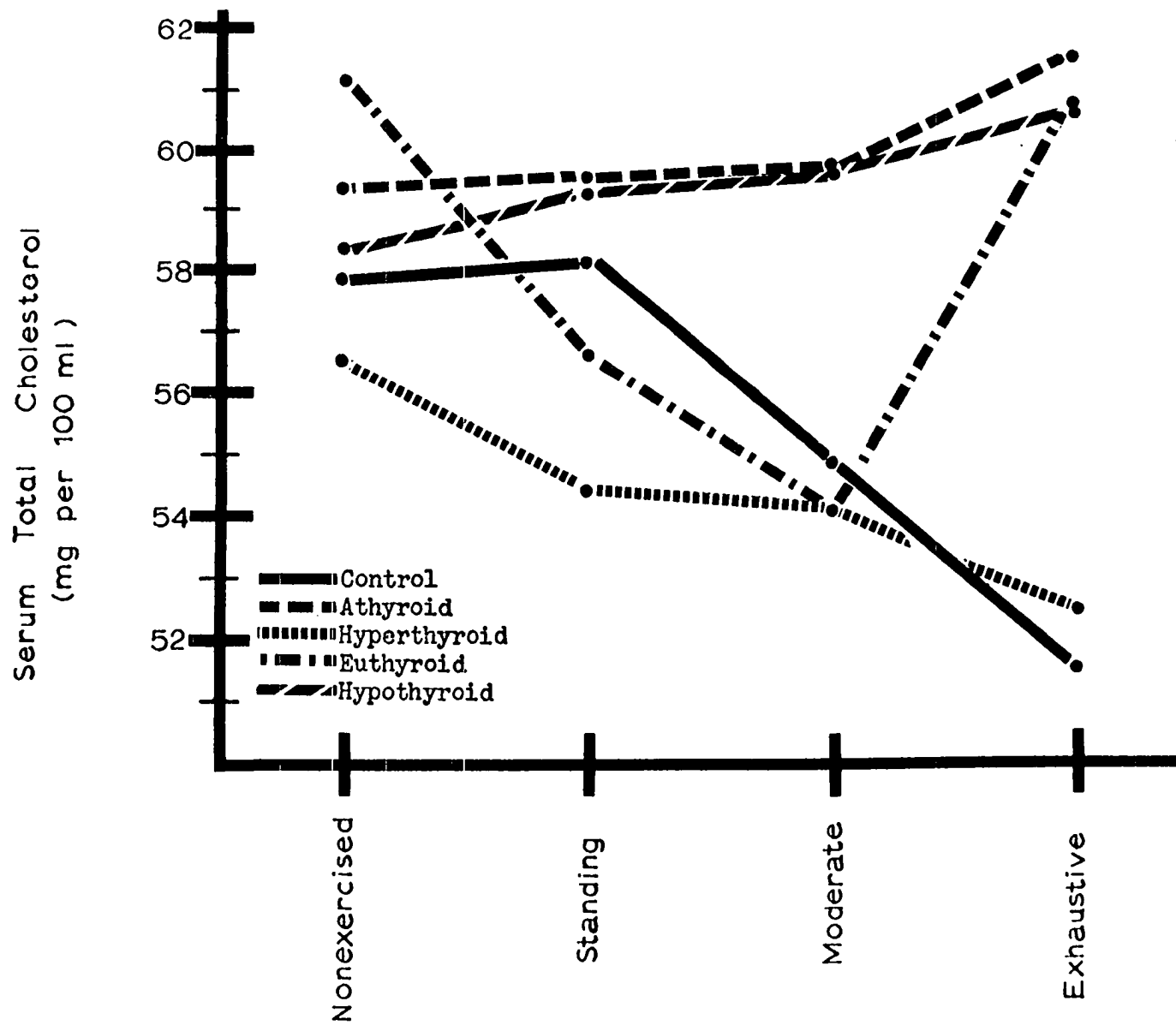
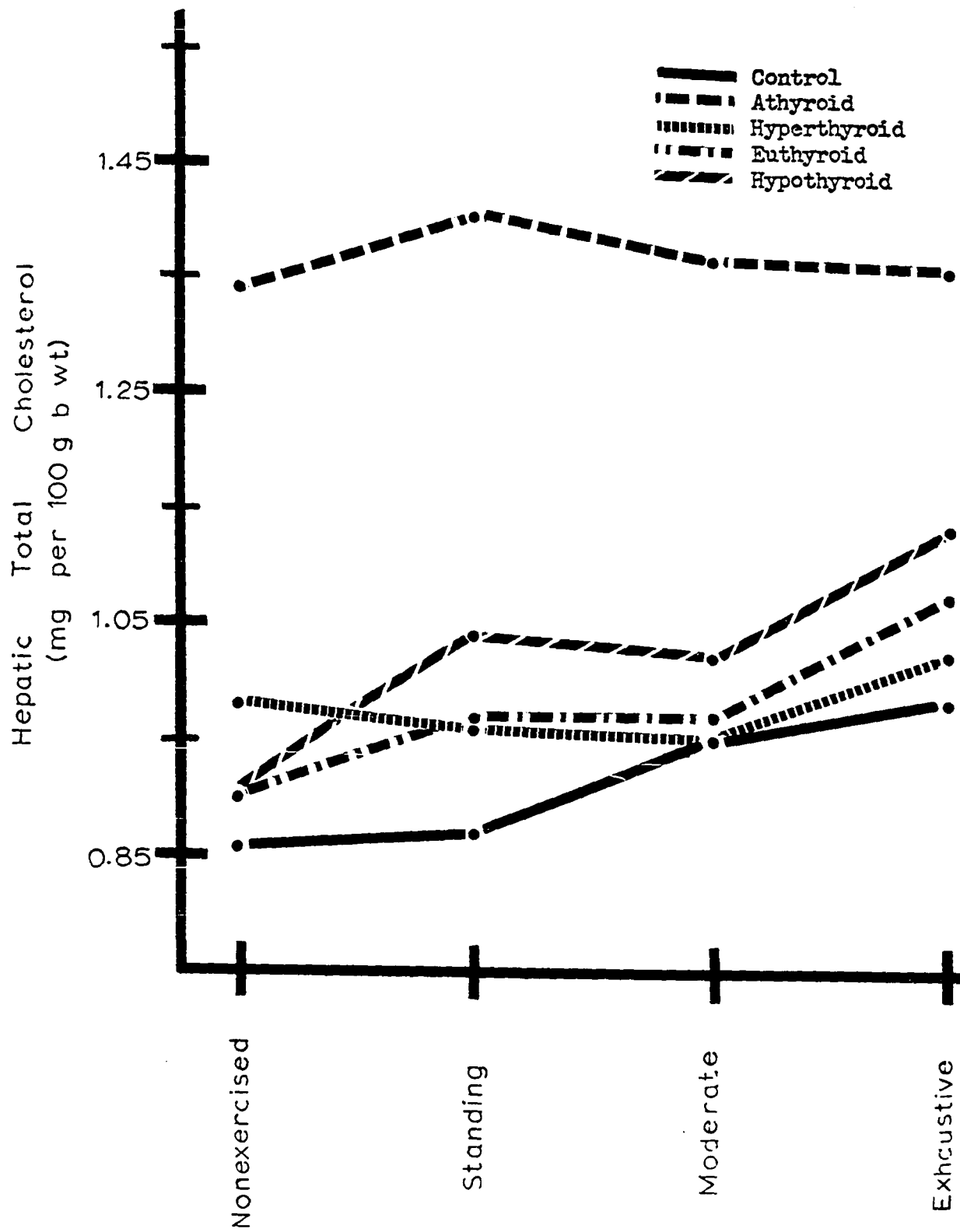


Figure 7. The influence of four levels of physical activity and five levels of thyroid status on hepatic total cholesterol concentration (milligrams per 100 grams body weight)



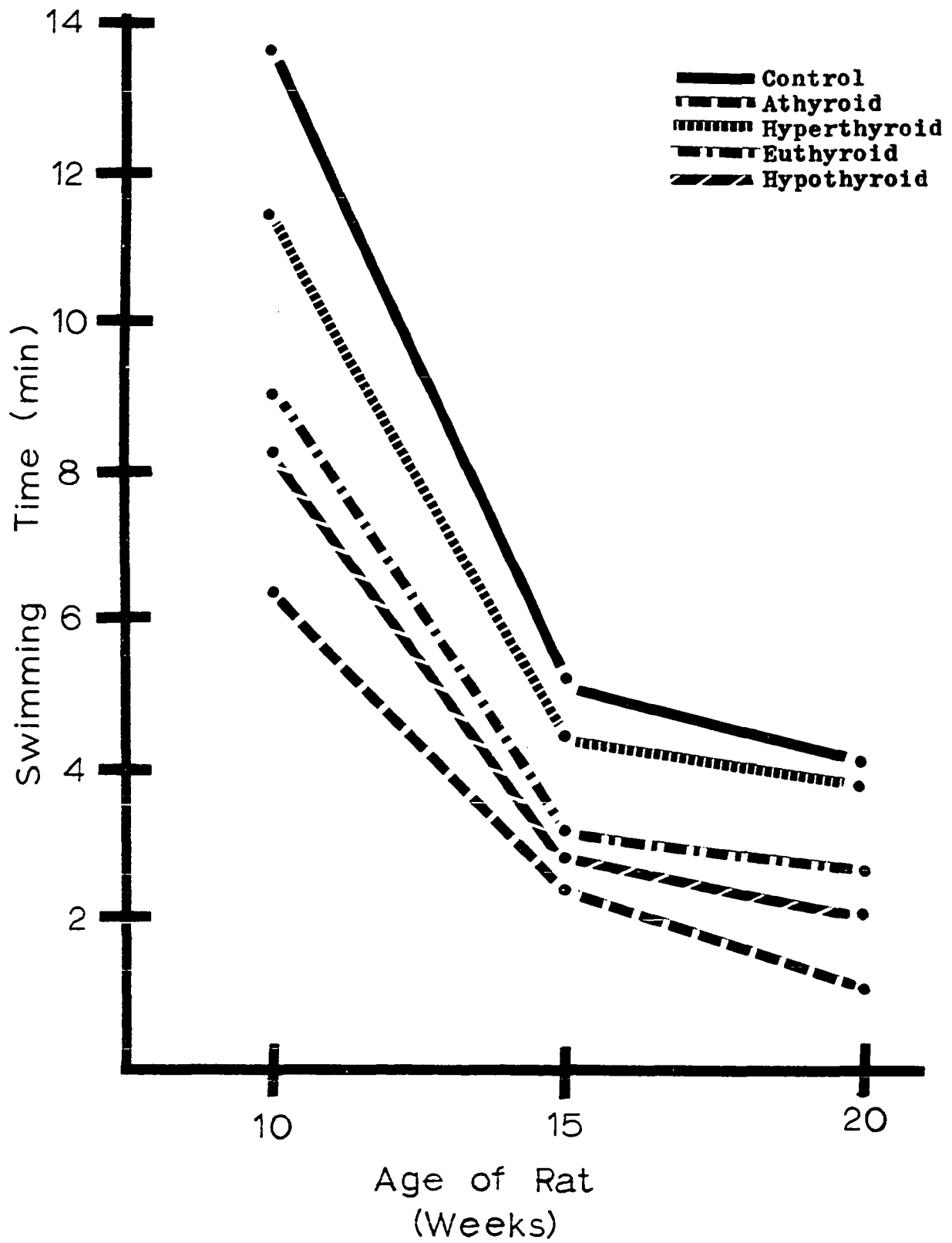


Figure 8. Swimming time of rats swum until exhausted

Procedure Used in the Preparation of  
L-Thyroxine Solutions<sup>1</sup>

I. Preparation of stock solution:

1. Weigh 36.50 mg L-thyroxine (Na-salt) on power paper (Glassine).
2. Transfer L-T<sub>4</sub> to 1.0 L flask. Wash adhering crystals into the flask with approximately 300 ml of 0.1 N NaOH.
3. Add about 900 ml distilled water. Mix well.
4. Add (drop by drop) approximately 1N HCl until a fine white precipitate results. Add enough HCl to adjust final pH of solution to about 5.0.
5. Bring final volume up to 1.0 L with distilled water and store in refrigerator.
6. When injecting L-T<sub>4</sub>, add enough NaOH solution to the desired quantity of injection solution (add drop by drop) until it is slightly alkaline. This will cause the white precipitate to dissolve and the solution will become clear again. In this form the solution of L-T<sub>4</sub> is in the active state. Concentration equals 3.5 µg L-T<sub>4</sub>/0.1 ml solution.

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<sup>1</sup>Procedure from Dr. Ralph Anderson, University of Missouri, Columbia, Mo., on July 12, 1968.



## II. Preparation of 1.0 and 0.5 microgram L-T<sub>4</sub> solutions:

1. 14.3 ml stock solution (active form) of L-T<sub>4</sub> is pipetted into 50 ml volumetric flask. Bring level of solution to mark by the addition of distilled water. Concentration equals 1.0 µg/0.1 ml solution.
2. 7.15 ml stock solution of L-T<sub>4</sub> is pipetted into 50 ml volumetric flask. Bring level of solution to mark by the addition of distilled water. Concentration equals 0.5 µg/0.1 ml solution.

### Procedure for Hepatic Total Cholesterol Determinations

This method was designed as a rapid and simplified technique for total hepatic cholesterol analysis adapted to the AutoAnalyzer.

#### Materials and methods

The instrument system consisted of the Technicon Auto-Analyzer Sampler II, proportioning Pump, 95°C Heating Bath, Colorimeter, and Recorder. Samples were run at a rate of 30/hr for greater accuracy compared to 40/hr. Absorbance was read at 550 mμ in a 15 mm tubular flowcell. Reagents used were the same as described in AutoAnalyzer Method File, Cholesterol N-24a (Technicon Instruments Corporation, 1965).

Hepatic tissue preparation: 250.0 mg hepatic tissue samples were randomly selected from different portions of the liver of each experimental animal. The 250.0 mg sample was placed in a 30 ml tissue grinder containing 9.75 ml isopropanol (2-propanol), and completely homogenized (Naito and Griffith 1971a). The homogenate was decanted into a tube and centrifuged at 35,000 rpm for 10 minutes. The supernate was poured into a centrifuge tube and centrifuged for a second time at 35,000 rpm for 5 minutes. The clear supernate was decanted into a culture tube; capped; and frozen at -20°C until analyzed with the AutoAnalyzer as described in the Auto-Analyzer Method File, Cholesterol N-24a (Technicon Instruments Corporation, 1965).